The Role of Fundamental Research and Biotechnology in Finding Solutions to the Global Problem of Antibiotic Resistance

Robert E. W. Hancock

From the Department of Microbiology and Immunology and the Canadian Bacterial Diseases Network, University of British Columbia, Vancouver, British Columbia, Canada

Resistance to antibiotics is becoming a major problem worldwide. Exacerbating this situation is the fact that few new antibiotics are in the development pipeline, and, indeed, no novel class of antibiotics has been introduced into medical practice in more than 20 years. It is proposed that the solutions to the problem of antibiotic resistance will be found only through fundamental research that will probably use biotechnology as a tool. A variety of novel approaches being utilized in university laboratories and biotechnology companies are outlined. Two approaches in particular, namely, Synsorbs and recombinant cationic peptide antimicrobials, that have been developed through the Canadian Bacterial Diseases Network, a national research consortium, are discussed.

One of the major problems with respect to antibiotic chemotherapy is that, despite the intensive research that is being conducted by the multibillion-dollar pharmaceutical industry, no novel chemical class of antibiotics has been discovered in the past 20 years [1]. Indeed, all recently introduced antibiotic compounds are permutations (i.e., improved versions) of preexisting compounds. Thus, a situation whereby bacteria can mutate known resistance mechanisms to combat these improved relatives of earlier antibiotics has been created, and it is not unusual for significant resistance to be observed even before introduction of such antibiotics into the clinic [2]. One global priority must therefore be to encourage the development of novel antimicrobials, which will result in diversification of the weapons available in the war against pathogenic bacteria.

Two of the major limitations of this objective are the high cost (~$300 million per new chemical entity) of drug development within pharmaceutical companies and the observation that many of the larger multinational companies have actually decreased their activities or even ceased to invest in the discovery of new antibiotics [3]. Does this mean that there are no prospects for the future? I believe that we must look to fundamental researchers and to the fledgling biotechnology industry for new solutions to the antibiotic resistance crisis, since these groups can provide the innovative approaches and the technological sophistication needed to achieve meaningful solutions.

Fundamental researchers usually work in universities and research institutions. However, two factors are of major concern: the lack of urgency attributed to the antibiotic resistance problem and the reduced investment in research on antibiotics by national granting bodies [3]; this reduced investment contrasts markedly with the investment in such areas of research as the treatment of cancer and heart disease and virology and biotechnology. This focus has meant that there are relatively few researchers worldwide with the capacity to perform excellent research on antibiotics. It is therefore essential that research on antibiotics be encouraged in various countries through their national research grant funding bodies by making such research a national priority for future funding.

One method of refocusing research efforts that is proving effective in Canada is the establishment of a national research consortium through the Network of Centres of Excellence (NCE) program [4]. The Canadian Bacterial Diseases Network (CBDN) is a research consortium that comprises 39 principal researchers from 12 universities and four governmental laboratories (i.e., a total of 240 research employees, of whom >100 are Ph.D. microbiologists) and an annual budget of >$10.3 million (60% of this money is currently provided by the consortium's 41 industry partners).

The objectives of CBDN are those of the NCE program, namely (1) to perform excellent fundamental research on bacterial diseases through the establishment of collaborative teams of researchers from across the country and (2) to speed the movement of novel antimicrobial compounds and technologies derived through this program from the university sector to industry.

To date, the success of this venture has been attested to by the 20 novel products and technologies that have been transferred to industry and by the unprecedented rate of productivity in terms of intellectual property (i.e., 58 patent filings). Indeed, the network has managed to take the novel therapeutic Synsorb-Pk (Synsorb Biotech, Calgary, Alberta, Canada) [5], designed to combat "hamburger disease" (hemolytic uremic syndrome), from conceptualization to clinical trials (currently, phase 3 trials) in <4 years; to date, the total investment by CBDN and its industry partner has been <$2 million.

The new biotechnology industry is the second important ingredient in the development of new antibiotics. Biotechnology companies, in contrast with most pharmaceutical companies, are typified by the following characteristics: strong recip-

Reprints or correspondence: Dr. Robert E. W. Hancock, Department of Microbiology and Immunology and the Canadian Diseases Network, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z3.

Clinical Infectious Diseases 1997;24(Suppl 1):S148–50

1058–4838/97/2401–0052$02.00

rocal linkages with the university sector, a far smaller number of employees and lower capitalization, a much lower average cost associated with product development (this cost currently averages ~$115 million), and a sophisticated research base. These characteristics all contribute to a lessening of internal bureaucracy and a bolder approach to product development. Although the potential for profitability of most biotechnology companies is indeed uncertain, the industry generated sales in excess of $7 billion in 1993.

It is of interest to note that although the biotechnology industry was largely built around the platform technology of recombinant DNA manipulation (genetic engineering), which itself was initiated in bacteria, this industry has only recently started to attack the problem of antibiotic-resistant microorganisms. This circumstance is reflected in the fact that only 15 biotechnology companies and their innovative approaches to "killer microbes" were listed in the 5 September 1994 issue of *Fortune* magazine. This figure probably represents about a fivefold underestimate of the number of companies that are specifically dedicated to overcoming antibiotic resistance problems, and we can anticipate that many more such companies will be established in the next decade.

The general approaches to creating novel products that are effective against antibiotic-resistant microbes are multiple but include (1) production of novel antimicrobial cationic peptides that mimic (and improve upon) the peptides that are used as natural antimicrobials by most forms of life [6]; (2) development of innovative, rational screening procedures for compounds that attack novel targets within the bacterial cell, based on simple colorimetric tests that can be applied to the search or screening for new antibiotics [1]; (3) investigation of the chemical basis for traditional antibacterial medicines (ethnopharmacology) [7]; (4) development of products that block the adherence of microbes or their toxins to tissues in the human body, thus preventing the tissue-specific growth of microbes or the effects of their toxins [5]; (5) rational drug design through the fitting of model chemicals into the crystal structures of the catalytic sites of key enzymes from bacteria [8]; (6) the screening of vast libraries of peptides (so-called combinatorial libraries that are produced chemically or by means of recombinant DNA technology) for those peptides capable of blocking key steps in bacterial pathogenesis [9]; (7) identification of novel enzymatic targets that are common to several pathogens, which, when inhibited, will result in blockage of bacterial growth; (8) the development of novel vaccines based on recombinant DNA technology (e.g., live oral *Salmonella* vaccines that are genetically engineered to produce antigens from other pathogens such as *Vibrio cholerae*) [10]; (9) development of products that block key steps of eukaryotic cell metabolism, which are parasitized by bacteria that invade and grow inside host cells (e.g., *Mycobacterium tuberculosis* and *Chlamydia trachomatis*); (10) development of cytokines, adjuvants, and other products that boost immune and/or nonspecific defenses against microbial infections [11]; and (11) an "antibiotic plus" strategy, in which compounds that are intended to be given together with existing antibiotics are designed to specifically counteract mechanisms of resistance to those antibiotics (the existing β-lactam and β-lactamase inhibitor compounds are an example of this strategy) [12].

Two brief examples of novel products, developed through CBDN, can be cited. The first, mentioned above, is Synsorb-Pk [5], which is designed to combat the *Escherichia coli* verotoxin that causes the nephrotoxic and occasionally lethal effects of hemolytic uremic syndrome in children; this disease is known more popularly as hamburger disease. Synsorb-Pk contains particles of diatomaceous earth (basically, the silicate skeletal structure of diatoms) that are conjugated to a trisaccharide that mimics the normal receptor for verotoxin in the gastrointestinal tract. Synsorb-Pk is given orally and binds toxin in the gastrointestinal tract, resulting in removal of toxin from the system via excretion and in prevention of the serious toxic sequelae associated with the binding to gastric cells and subsequent uptake of toxins. Such a strategy is relatively simple, inexpensive to develop, and easily extrapolated to other gastrointestinal (diarrheal) diseases.

A second strategy being pursued by several biotechnology companies involves small cationic peptides [6]. Examination of the literature revealed that polycationic peptides are ubiquitous in nature; >150 small cationic antibacterial peptides have been observed in organisms including bacteria, plants, insects, amphibians, crustaceans, mammals, and humans. In plants and insects, such peptides represent the major inducible defense against bacteria and other microorganisms, whereas in mammals and humans they are known to be important factors in the arsenal of neutrophils (defensins are the major proteinaceous molecule in neutrophils), and other peptides are suspected of having a major role in defense of mucosal surfaces.

To investigate the therapeutic potential of these peptides, we devised methods of producing them in bacteria by means of recombinant DNA procedures [13]. The method of choice involves production of these peptides by fusion protein technology, wherein a four-part fusion protein is encoded by a plasmid in *E. coli* or *Staphylococcus aureus*. This fusion protein contains (from the N- to C-termini) an affinity binding region (for ease of purification), an anionic stabilizing fragment (to neutralize the cationic peptide portion and prevent its bactericidal action and cleavage by bacterial proteases), and a cationic antimicrobial peptide. The latter three regions are encoded by synthetic DNA, and their sequence can be changed rather easily, permitting a large range of cationic peptides to be produced by this technology. After purification, a peptide produced by recombinant DNA technology is indistinguishable from one made by protein chemical means, except that it costs only 5% as much to produce.

With use of this technology, my colleagues and I have produced a variety of different peptides including a human defensin, α-helical hybrids of moth cecropin and bee venom melittum (MBI.27 and MBI.28), indolicidin, bactenicin, and

apidaecin. The α-helical class was studied in detail [14]. These peptides were shown to access the self-promoted uptake pathway in *Pseudomonas aeruginosa* (and *Enterobacter cloacae*), and they demonstrated rapid killing even at their MIC when compared with all other known classes of antibiotics. These peptides killed most clinically problematic gram-negative and gram-positive organisms at reasonable concentrations, were equally effective against parental strains and antibiotic-resistant mutants, and engendered no resistance themselves during in vitro experiments. They also demonstrated "enhancer" activity in that they breached the outer membrane permeability barrier of *P. aeruginosa* and enhanced the uptake of lysozyme and certain antibiotics.

Cationic peptides bound tightly to endotoxin and neutralized its ability to induce TNF in macrophage cell lines and its lethal action against galactosamine-sensitized mice. In addition to their antibiotic and antiendotoxic activity, some of the peptides demonstrated useful antifungal activity. My colleagues and I have proposed that such cationic peptides are potential alternatives to classic antibiotic therapy for nosocomial infections as well as pseudomonal lung infections in patients with cystic fibrosis.

I believe that innovative approaches such as those described above will provide us with novel products that can contribute to the future management of bacterial diseases. However, governments must work to create the funding and regulatory environments that will encourage research on this problem.

References

1. Silver L, Bostian K. Screening of natural products for antimicrobial agents. Eur J Clin Microbiol Infect Dis **1990**;9:455–61.
2. Neu HC. Infection problems for the 1990s—do we have an answer? Scand J Infect Dis Suppl **1993**;91:7–13.
3. Travis J. Reviving the antibiotic miracle. Science **1994**;264:360–2.
4. Hancock REW, McCurry TE. Canadian Bacterial Diseases Network: a new approach to university-industry relationships. Clin Invest Med **1993**;16: 306–13.
5. Armstrong GD, Rowe PC, Goodyer D, et al. A phase I study of chemically synthesized verotoxin (Shiga-like toxin) Pk-trisaccharide receptors attached to chromosorb for preventing hemolytic-uremic syndrome. J Infect Dis **1995**;171:1042–5.
6. Hancock REW, Falla TJ. Antimicrobial peptides: broad spectrum antibiotics from nature. Clin Microbiol Infect **1989**;1:226–9.
7. Farnsworth NR. Ethnopharmacology and drug development. Ciba Found Symp **1994**;185:42–51.
8. Seydel JK. Modern strategies in the design of antimicrobial agents. In: Jackson GG, Schlumberger HD, Zeiler HJ, eds. Perspectives in antiinfective therapy. Braunschweig, Germany: Vieweg and Sohn, **1989**: 21–8.
9. Blondelle SE, Takahashi E, Houghten RA, Perez-Paya E. Rapid identification of compounds with enhanced antimicrobial activity by using conformationally defined combinatorial libraries. Biochem J **1996**;313: 141–7.
10. Dougan G. Molecular characterization of bacterial virulence factors and the consequences for vaccine design. J Gen Microbiol **1989**;135: 1397–406.
11. Roilides E, Pizzo PA. Modulation of host defenses by cytokines: evolving adjuncts in prevention and treatment of serious infections in immunocompromised hosts. Clin Infect Dis **1992**;15:508–24.
12. Livermore DM. Determinants of activity of β-lactamase inhibitor combinations. J Antimicrob Chemother **1993**;31(suppl A):9–21.
13. Piers KL, Brown MH, Hancock REW. Recombinant DNA procedures for producing small antimicrobial cationic peptides in bacteria. Gene **1993**; 134:7–13.
14. Piers KL, Brown MH, Hancock REW. Improvement of outer membrane-permeabilization and lipopolysaccharide-binding activities of an antimicrobial cationic peptide by C-terminal modification. Antimicrob Agents Chemother **1994**;38:2311–6.