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Disruptive innovations: new anti-infectives in the age of resistance

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Abstract

This special issue of Current Opinion in Pharmacology is concerned with new developments in antimicrobial drugs and covers innovative strategies for dealing with microbial infection in the age of multi-antibiotic resistance. Despite widespread fears that many infectious diseases may become untreatable, disruptive innovations are in the process of being discovered and developed that may go some way to leading the fight-back against the rising threat. Natural products, quorum sensing inhibitors, biofilm disruptors, gallium-based drugs, cyclodextrin inhibitors of pore-forming toxins, anti-fungals that deal with biofilms, and light based antimicrobial strategies are specifically addressed. New non-vertebrate animal models of infection may facilitate high-throughput screening (HTS) of novel anti-infectives.

A new chapter in the book of infectious diseases

The U.S. Surgeon General William H Stewart remarked in a 1969 statement to congress 'The time has come to close the book on infectious disease' [1]. The golden era of antibiotic discovery that had lasted from 1950 until 1970 was coming to an end. Even at that time some scientists were skeptical at such a bold statement, and doubted that a single technology could win the war against infectious disease, but then antibiotic resistance was more of a test-tube concept under laboratory investigation, rather than a major public health concern. The sequence of events over the last half-century that has led to the present 'era of resistance' has been rapid. Many conventional anti-infectives have become ineffective; a variety of complex and sometimes overlapping antimicrobial resistance mechanisms has been unraveled; thousands of human lives have been lost due to emerging and re-emerging microbial pathogens; and there is now worldwide awareness for the need to add new chapters to the book of infectious diseases. Nobel laureate Joshua Lederberg stated [1] that technology will never win this war permanently and we must be satisfied to merely stay one step ahead of the pathogens; thus, the result of this evolutionary race between the host and the pathogen is the constant quest for novel anti-infective technologies and innovative drug discovery approaches.

From ‘superbugs’ to the ‘end of the antibiotic era’

At the present time multidrug resistant microbes account for the majority of nosocomial and community acquired infections and represent an exponentially growing threat to human health. These organisms have now dominated the scientific literature and have given rise to the term ‘superbugs’ [2]. Infectious disease in the 21st century is again at the epicenter of a global dialogue capturing the attention of academics, governments, public health officials, and the general public alike. Each year over 13 million deaths worldwide that are attributed to the emergence of new infectious diseases or to the re-emergence of diseases previously controlled and sometimes forgotten, can also be attributed to widespread multidrug resistance (MDR). Dynamic shifts in global socio-economic trends, environmental ecological factors, and changes in the microorganisms themselves have resulted in heightened public health concern with an emphasis on antimicrobial resistance [3]. Controlling this challenge in the new millennium requires rational, as well as unconventional, antimicrobial drug discovery efforts that are aligned with, and help to foster public awareness with an emphasis on combating the emerging problem. The list includes established threat organisms such as the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter species*) [4] as well as emerging and re-emerging pathogens such as carbapenem-resistant *K. pneumoniae* (CRKP), the New Delhi metallo-beta-lactamase containing Enterobacteriaceae, MDR and extensively drug-resistant (XDR) strains of *Mycobacterium tuberculosis* (MDR-TB and XDR-TB) and non-tuberculous mycobacterial MDR resistance, *Candida albicans* and pathogenic fungi. The inappropriate, unnecessary or excessive use of antibiotics in human therapy, as well as their use in agriculture, aquaculture and livestock industries, select over time for resistant strains and appear to be the key contributors to the emergence of antimicrobial resistance. This reality has given rise to the discipline called ‘antibiotic stewardship’ [5]. In hospitals, resistant bacteria can survive for a prolonged time and can cause epidemics, for example, in intensive care units. The risk of infection increases with the amount of time spent in the hospital. Some pathogenic bacteria in intensive care units, for example, *A. baumannii*, *P. aeruginosa*, *K. pneumoniae* have been described as ‘pan-resistant’. This gloomy reality is often described as the ‘end of the antibiotic era’ [6].

Industry versus academia

A large fraction of the key player large companies in the pharmaceutical arena have abandoned their anti-infective research programs in the recent past. This trend is underlined by the observation that it is easier to name the few companies that still retain a program, even if it is not prioritized, than to enumerate those who have abandoned their anti-infective research. The reasons for the de-emphasis on industrial development of new antimicrobials can be categorized as perceptual (market saturation, scarcity of new drug targets, increasing difficulty in gaining regulatory approval), as practical (the disinclination to embark on complex natural product discovery and development research programs, increased time and cost required for HTS efforts in genomics, proteomics, combinatorial synthetic chemistry, development time required for clinical trials) and as conceptual (‘merger-mania’). As the need to combat resistance persists and evolves, the scientific community has come up with an array of different approaches and models that may be attractive and tempting alternatives to answer fundamental discovery questions [7]: Firstly, is there still a role for target-based antibiotic discovery? Secondly, should old targets be grouped by inhibitor scaffold or revitalized? Thirdly, is there promise for new targets by exploiting the microbial ‘phenotype’? Finally, are there reliable and reproducible animal models to link discovery and development?

The world of antibiotic drug discovery and development is driven by the necessity to overcome antibiotic resistance in common Gram-positive and Gram-negative pathogens. However, the lack of Gram-negative activity among both recently approved antibiotics and compounds in the developmental pipeline is a general trend, despite the fact that the plethora of covered drug targets are well-conserved across the bacterial kingdom.

The riddle of antimicrobial resistance

Several mechanisms have evolved in microorganisms which confer antimicrobial resistance [8]. These mechanisms can either chemically modify the antimicrobial agent, render it inactive through physical removal from the cell, or modify the target site so that it is not recognized by the antimicrobial. Resistance may be an inherent trait of the organism (e.g. a particular type of cell wall structure) that renders it *naturally resistant*, or it may be *acquired* by means of mutation in its own DNA or acquisition of resistance-conferring DNA from another source. This analysis may only be the tip of the resistance iceberg as recent intensive efforts have revealed. The actual mechanisms are more complex with numerous interacting pathways expanding beyond conventional microbiological structural and physiological (virulence and pathogenicity) determinants, incorporating also host defense elements and host genotypes. Efflux mechanisms for example are broadly recognized as major components of resistance to many classes of antimicrobials, as well as chemotherapeutics. Efflux occurs due to the activity of membrane transporter proteins widely known as multidrug efflux systems (MES). MES are implicated in a variety of physiological roles other than drug efflux, and identifying their natural substrates and inhibitors is an active and expanding research discipline. A number of structurally and functionally diverse compounds act as substrates or modulators of efflux systems. However, only a few are appropriate candidates for clinical use as MDR reversal agents [9]. Dual treatment with efflux pump inhibitors (EPIs) in conjunction with chemotherapeutics is an emerging treatment strategy to circumvent MDR in cancer. An array of discovery efforts in academic and industrial settings has yielded a number of promising EPIs in a series of pathogenic systems. The concept of enhancing the utility of antimicrobials by employing EPIs is an appealing concept, although translation of lead EPIs into clinically useful compounds remains a challenge. The role of efflux systems in microbial physiology is far more complicated than merely a resistance mechanism to antimicrobials. The predominant type of MES in Gram-negative bacteria are resistance nodulation cell division (RNDs) efflux systems that have been implicated in a series of virulence and pathogenicity pathways and phenotypes. It has been suggested that expression of efflux systems contributes to biofilm persistence and quorum sensing pathways. Efflux is also a key component in the social (intercellular) interaction in both synthetic and natural microbial populations. These interactions are considered important determinants of antibiotic resistance and tolerance. Multidrug tolerance has been attributed to *persister cells*, specialized non-dividing survivor cells protecting bacterial populations from antibiotic killing, implicated in biofilms, and chronic and recurrent infections [10]. Persisters may survive challenge with antibacterials by virtue of their metabolic inactivity. Eradication of these cells is currently challenging because the exact mechanisms of persister formation are still unclear. The current opinion on the role of persisters suggests that the following factors are important: firstly, heterogeneity as each bacterial population may contain a diverse collection of persisters, secondly, direct implication in the path from antibiotic tolerance to resistance, thirdly, the utility of metabolic stimuli in persister killing by aminoglycosides through generation of a proton-motive force which facilitates aminoglycoside uptake, and finally, efflux induction (through oxidative stress for example) leads to increased numbers of multidrug-tolerant persisters.

Natural antimicrobials

The first paper by Bologa *et al.* in the special issue covers advances in the development of natural product antimicrobials. Natural products and their analogs continue to play a prominent role in medicine, accounting for two-thirds of new antibacterial therapies approved from 1980 to 2010 as well as several antibacterials currently in clinical trials [11]. The efficacy of natural products as antibacterial agents likely stems from the fact that they have been honed by millennia of evolutionary processes to be bioactive, thereby giving the organisms producing them a selective advantage in the environment. Rapid advances in our understanding of and ability to manipulate natural product biosynthesis pathways, increasingly driven by informatics, chemical biology, and synthetic biology, have introduced potential new classes of antimicrobial drugs. Most of these are still at the stage of bench research (e.g. Tetarimycin A, an MRSA-active antibiotic identified through induced expression of environmental DNA gene clusters, and arylomycin A2) but they hold promise for speedy progress to the patient bedside.

Quorum sensing inhibitors

Zhu and Kaufmann propose a novel antimicrobial approach based upon inhibiting or 'quenching' bacterial quorum sensing. Interference with quorum sensing signaling might offer new avenues to prevent and/or treat bacterial infections via inhibition of virulence factor expression and biofilm formation [12]. Prophylactic quorum quenching approaches have demonstrated efficacy *in vivo*. Quorum sensing antigens might warrant inclusion in microbial vaccination strategies. Combination of quorum quenching agents and antibiotics might offer synergies. Neutralization of quorum sensing molecules may restore proper host immune function. While many inhibitors of quorum sensing signaling have been described, only a few have been evaluated *in vivo* and up to now none has been clinically developed.

Biologically inspired strategies for combating bacterial biofilms

Blackledge *et al.* discuss a range of strategies to combat bacterial biofilms, focusing firstly on small molecule interference with bacterial communication and signaling pathways, including quorum sensing and two-component signal transduction systems. Enzymatic approaches to the degradation of extracellular matrix components to effect biofilm dispersal are covered. Both these approaches are based upon non-microbicidal mechanisms of action, and thereby do directly exert selective pressure on the bacteria to develop resistance. Anti-biofilm approaches have the potential to be combined with conventional antibiotics to produce a synergistic technique for the eradication of biofilm based bacterial infections.

Gallium based anti-infectives

Kelson *et al.* describe a novel antimicrobial approach based on the trivalent metal, gallium. Microbes have evolved elaborate iron-acquisition systems to sequester iron from the host environment using siderophores and heme uptake systems. Since gallium(III) is structurally similar to iron(III), except that it cannot be reduced under physiological conditions, gallium can behave as an iron analog, and thus an antimicrobial. Because Ga(III) can bind to virtually any complex that binds Fe(III), both simple gallium salts, and complexes between Ga(III) and siderophores or heme derivatives are potential carriers to deliver Ga(III) to the microbes. Gallium complexes simple gallium salts such as gallium nitrate, maltolate and simple Gasiderophore complexes such as gallium citrate have shown good antibacterial activities. Gallium citrate exhibits broad activity against many Gram negative bacteria at MICs of ~1–5 µg/mL, shows strong anti-biofilm activity, low drug resistance, and efficacy *in vivo*.

Cyclodextrin derivatives (CD) as anti-infectives

Karginov describes the use of CD to inhibit pore-forming proteins that are potent virulence factors produced by some pathogenic bacteria. Highly efficient selection of potent inhibitors was achieved since persubstituted cyclodextrins were found to possess the same symmetry as the target pores. Inhibitors of several bacterial toxins produced by *B. anthracis*, *S. aureus*, *C. perfringens*, *C. botulinum* and *C. difficile* were identified in a library of ~200 CD. They demonstrated that multi-targeted inhibitors could be found using this approach that may be utilized for the development of broad-spectrum drugs against various pathogens.

Anti-fungal therapy with an emphasis on biofilms

Pierce *et al.* discuss the increasing need for novel anti-fungal drugs. Since fungi are eukaryotic organisms there are a limited number of targets for anti-fungal drug development compared to prokaryotic or viral pathogens. Azoles, polyenes and echinocandins constitute the mainstay of anti-fungal therapy for patients with life-threatening mycoses. One of the main factors complicating anti-fungal therapy is the formation of fungal biofilms that provide resistance to most anti-fungal agents. A better understanding of fungal biofilms may provide for new opportunities for the development of urgently needed novel anti-fungal agents and strategies. Special emphasis is placed in combinatorial treatments targeting fungal biofilms (Hsp90 and calcineurin inhibitors is a notable example) drug screening and repurposing, catheter lock solutions (for catheter-associated infections).

Light based-antimicrobial strategies

Yin *et al.* cover a group of antimicrobial approaches, which all rely on light to deliver the killing blow. Although ultraviolet light has long been used as a germicidal treatment, its use as a therapeutic for infections has not been studied until recently when it was realized that the possible adverse effects to host tissue were relatively minor compared to its high activity in killing pathogens. Photodynamic therapy employs the combination of non-toxic dyes with harmless visible light that together produce highly destructive reactive oxygen species. When cationic dyes are used to provide microbial selectivity, this approach can be used for treating both superficial and even deep-seated infections using fiber optic delivered light. Many microbial cells are highly sensitive to killing by blue light (400–470 nm) due to accumulation of naturally occurring photosensitizers such as porphyrins and flavins. Near infrared light has also been shown to have antimicrobial effects against certain species. Clinical applications of these technologies include skin, dental, wound, stomach, nasal, toenail and other infections which are amenable to effective light delivery.

Drosophila melanogaster as a model host

The last two papers in the Special Issue cover the use of non-vertebrate hosts for drug discovery of new antimicrobials. The first contribution by Tzelepis *et al.* concerns the fruitfly *D. melanogaster*. Drug screening in *Drosophila* offers to fill the gap between *in vitro* and mammalian model hosts by eliminating compounds that are toxic or have reduced bioavailability and by identifying others that may boost innate host defence or selectively reduce microbial virulence in a whole-organism setting. *Drosophila* has significant similarity with humans and a short life cycle of ~10 days from egg to sexually mature adult as compared to the ~2 months of mice. Large numbers of flies can be propagated quickly, since tens of females can produce hundreds of offspring within 2 weeks. Due to its small size of 2 mm in length thousands of flies can be contained in a space that would normally fit <10 mice. In addition, fly food is usually made of grocery store ingredients such as cornmeal, yeast and sucrose, thus the cost of maintenance is quite low. Moreover, there are no ethical concerns or regulated protocols for its use in biomedical research. Importantly, drugs can be

mixed in the fly food or administered by injection and only small quantities of drugs are required for testing.

***Caenorhabditis elegans* for anti-infective drug discovery**

The final contribution by Arvanitis *et al.* also covers another non-vertebrate host for drug discovery, in this case the nematode worm *C. elegans*. This alternative host has been used to identify traditional microbicidal agents, including antihelminthic compounds, as well as novel agents that attenuate microbial virulence or enhance the host's immune response. Its amenability to high-throughput automated screening can allow for the detection of bioactive products among thousands of tested substances.

Conclusions and future perspectives

The early chemotherapeutic antimicrobials originated from synthetic chemistry (e.g. dye research), as embodied by sulfa drugs, in the early 1930s. Then followed the great era of antibiotic discovery, that quickly led to predictions of the end of infectious disease as a significant clinical problem. Increasing antibiotic resistance over the last 50 years turned this rosy outlook to a gloomy prognosis of untreatable infections waiting to pounce on any unsuspecting hospital patient. The articles in this special issue highlight recently developed disruptive innovations in the discovery of anti-infectives and development of interventions to combat infectious disease. They highlight the identification of new drug targets, key pathways and resistance mechanisms that will help develop new treatments or improve existing ones. We thank all the authors for their excellent contributions and the publishing team (Pien van Spijker and Fred Kop) for their help during the past 12 months in compiling this issue. We hope that readers will find in this issue new avenues of research in the fight against multidrug resistant pathogens.

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Biographies

George P Tegos is an assistant professor at the Department of Pathology School of Medicine at the University of New Mexico affiliated with the Center of Molecular Discovery and a Visiting Scientist at the Wellman Center for Photomedicine at Massachusetts General Hospital, Harvard Medical School. He received his PhD from University of Ioannina, Greece. He completed postdoctoral fellowships in Molecular Microbiology (Antimicrobial Discovery Center at Northeastern University, 2001–2003) and Translational Therapeutics in Dermatology (Wellman Center for Photomedicine, Harvard Medical School at Massachusetts General Hospital, 2003–2006). His research interests lie in the areas of drug discovery and development of antimicrobial strategies with emphasis in photodynamic therapy for infections, multidrug efflux systems as well as virulence and microbial pathogenesis. His research program is supported by the US National Institutes of Health, the Department of Defense (DOD-DTRA) and the Clinical and Translational Science Center at the University of New Mexico (CTSC-UNM). He has been an EU Marie Curie fellow in Biotechnology and a recipient of the Massachusetts Technology Transfer Center (MTTC) award in Antimicrobials. He has published over 65 peer-reviewed articles, over 80 conference proceedings, book chapters and International abstracts, served as an ad hoc reviewer for a variety of journals and funding organizations in US, Europe and Asia and has delivered more than 40 invited presentations.

Michael R Hamblin is a principal investigator at the Wellman Center for Photomedicine at Massachusetts General Hospital, an Associate Professor of Dermatology at Harvard Medical School and is a member of the affiliated faculty of the Harvard-MIT Division of Health Science and Technology. He was trained as a synthetic organic chemist and received his PhD from Trent University in England. His research interests lie in the areas of photodynamic therapy (PDT) for infections, cancer, and heart disease and in low-level light therapy (LLLT) for wound healing, arthritis, traumatic brain injury and hair regrowth. He directs a laboratory of around a 16 post-doctoral fellows, visiting scientists and graduate students. His research program is supported by NIH, CDMRP, USAFOSR and CIMIT among other funding agencies. He has published 232 peer-reviewed articles, over 150 conference proceedings, book chapters and International abstracts and holds eight patents. He is Associate Editor for seven journals, on the editorial board of a further 12 journals and serves on NIH Study Sections. For the past 9 years Dr Hamblin has chaired an annual conference at SPIE Photonics West entitled ‘Mechanisms for low level light therapy’ and he has edited the nine proceedings volumes together with two other major textbooks on PDT. He has several other book projects in progress at various stages of completion. In 2011 Dr Hamblin was honored by election as a Fellow of SP.