would undoubtedly involve others, more often than not their elders, making recommendations.

My aim is not to propose a replacement for the present system, but to highlight a need for a wider discussion of possible bias. At present no one can halt the progression of age, and all must live with its consequences.

Food for thought for those involved in peer evaluation!

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Raymond C. Rowe
Pharmaceutical and Analytical R&D
AstraZeneca
Alderley Park
Macclesfield
Cheshire
UK SK10 2NA
tel: +44 (0)1625 513112
fax: +44 (0)1625 512381
e-mail: ray.rowe@astrazeneca.com

Can smart bullets penetrate magic bullet-proof vests?

Jack A. Heinemann, Dept of Plant and Microbial Sciences, University of Canterbury, Christchurch, New Zealand and Norwegian Institute of Gene Ecology, Tromsø, Norway

Ehrlich's concept of a 'magic bullet' has long been a powerful focusing metaphor for the technology of treating infectious diseases. Magic bullets are usually considered to be agents with low toxicity to humans, high toxicity to microbes and with the ability to be delivered at efficacious concentrations to the site of infection. Ehrlich's idea has become ubiquitously associated with antibiotics and other modern antimicrobial agents: chemotherapeutics that unfortunately fall short of his grand concept. Rather than attempt to wrestle apart the magic bullet and antibiotic, here I discuss 'smart' bullets, that is, hypothetical agents that could build on the qualities of the magic bullet that have not been successfully designed into conventional antibiotics.

Antimicrobial agents have always dominated the modern infectious-diseases drug-discovery programme for good reason: it is self-evident that patients are rid of infections concomitantly with the organisms that cause the disease. These agents are not smart bullets, however, because they are toxic to both disease-causing and benign microbes. More importantly, they contribute directly to the evolution of resistance. Are antimicrobial agents the best approximation of the smart bullet we can achieve? Are we incapable of finding drugs that will treat infectious disease without incurring the ecological side-effects common to antibiotics, that of killing normal flora and selecting resistant pathogens?

Despite some 50 years of successful application of antimicrobial agents, their primacy in infectious disease management is increasingly being questioned[1–3]. Many researchers are asking whether drugs with alternative properties could be developed that could productively augment the use of antimicrobial agents[4,5].

Although an emphasis on the quick elimination of pathogens in acute infections is justified, there is also reason to develop drugs according to other priorities. Drugs developed for long-term use and longer-term efficacy, possibly at the expense of rapid effect, could enhance our other disease-management tools. Disappointingly few alternative concepts to antimicrobial agents are being discussed and even fewer technologies and proven alternatives have been offered. With the exceptions of some vaccines directed against the virulence determinants of pathogens, rather than the viability of the microorganism, hygienic practices and most vaccines are still effectively antimicrobial.

Although there are alternative agents in development, the number is small and the rationale for their development is not broadly understood. I would argue that a change in our understanding of microbial evolution is necessary to fully appreciate why conventional antimicrobial agents have limited lives, and why industry and academia should be trying harder to replace them.

A better understanding of evolutionary mechanisms will allow us to fruitfully invest in new kinds of drug-discovery strategies for truly new kinds of drugs. The truly novel drugs discussed here

1359-6446/01/$ – see front matter PII: S1359-6446(01)01857-8

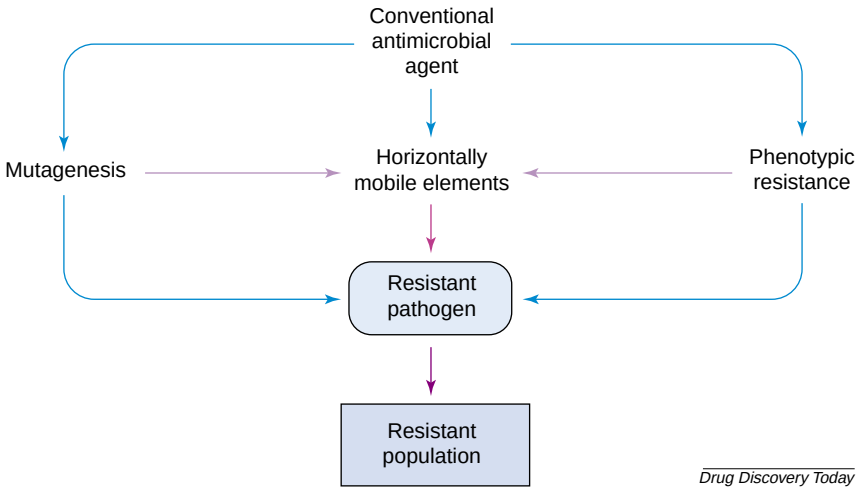

Figure 1. Effect of therapeutics on the evolution of resistance. Traditionally, the analysis of resistance has been on how antibiotics protect rare resistant individual bacteria until they grow into populations of resistant pathogens in the absence of competition from sensitive microorganisms. A full evaluation of resistance evolution and drug development must consider how resistance arises in individual pathogens. The specificity of antimicrobial agents can preserve non-target biochemical activities, some of which can be involved in inter-organism gene transfer. These pathways continue to function in the presence of the drugs just as well, sometimes even better, than in the absence of drugs. Other properties of chemotherapeutics might enhance gene transfer by transformation. Some drugs increase the basal mutation rate increasing the probability of exposed bacteria to develop genotypic resistance. This process could be relevant to populations of pathogens even if the genotypically resistant cell dies. If the mutation was on a horizontally mobile element within the cell it could still be spread by infectious exchange. By mimicking signals that induce alternative physiological states, some drugs induce phenotypic resistance to themselves and other drugs. By increasing the infection time, phenotypically resistant cells are more likely to acquire genotypic resistance by inter-organism gene transfer. They might also produce more offspring, increasing the probability of acquiring genotypic resistance through mutation.

might be thought of as 'smart' bullets, rather than the conventional 'magic' bullets that so 'successfully' select pathogens with magic-bullet-proof vests.

The mystery of horizontal gene-transfer

In addition to the many mathematical treatments of microbial evolution[6], there is now laboratory evidence[7] that phenotypic resistance is, at least in its earliest stages, a side effect of natural selection acting specifically on the evolution of individual genetic units, rather than on microorganisms *per se*[6,8–10]. No one is surprised by the story that bacteria exchange antibiotic resistance genes by a process called horizontal gene-transfer. However, they should be: there is no theoretical or empirical basis for expecting resistance to have evolved by horizontal gene-transfer[11]. The mechanisms of gene transfer are described with molecular precision, and the benefits to an organism that receives a new resistance gene are obvious; thus, perhaps, most of us have been comfortable with assuming that the effects of gene transfer (benefit to the microbe) were the cause of resistance genes evolving on the vectors that mediate transfer (Fig. 1).

By contrast, few virologists would suggest that retroviruses exist to transfer genes between humans because some of those transferred genes could be of benefit to us! Why then, do we think that the viruses of bacteria and other such infectious genetic agents, such as plasmids, transposons, integrons and conjugative transposons, have evolved to create genetic diversity in bacteria?

With this in mind, we must ask: (1) why do infectious genetic agents of bacteria carry antibiotic resistance genes, (2) what causes these particular genes to evolve on such vectors so frequently, and (3) why has this only occurred since the human use of antimicrobial agents? The answers to these questions should provide us with clues for designing smart bullets: anti-infective agents with limited potential to select for resistance and the horizontal transfer of resistance genes.

Potential smart bullets for treating infectious diseases

Manipulating virulence

Agents that manipulate the virulence of would-be pathogens rather than their reproductive ability *per se* would not be expected to select horizontally mobile resistance. The idea for drugs of this class originates with others[12], albeit proposed for different reasons. Basic research is producing some potential drug sources, from peptide nucleic acids (PNAs)[13] to drugs that might quench cell-density-dependent signals that induce virulent phenotypes[3,14,15]. Several other possible technologies were discussed at the *2001 International Symposium on Progress in Drug Discovery and Development Sciences* conference (17–19 January 2001, Bangalore, India), which was sponsored by AstraZeneca India and Research Foundation India, and was held at the Indian Institute of Science (Bangalore, India).

Glen Armstrong of the University of Alberta (Edmonton, Canada) described strategies for interfering with the binding of either microbes, or their toxins, to the tissues they target when causing disease, thus preventing an early step in their pathogenesis. Armstrong designs carbohydrates that compete with microbial adhesions and toxins for binding to their cellular receptors. Thus, only pathogenic strains of particular microbes would be

targeted by his strategy. Moreover, the therapeutic carbohydrate need not be toxic to the microorganism and, therefore, might not select for resistance. As a potential bonus, mutations in adhesions or toxins that alter their binding characteristics might also attenuate virulence. Therefore, a desirable outcome of developing receptor mimics might be to select for benign commensals rather than resistant pathogens.

Lars G. Burman of the Swedish Institute of Infectious Diseases Control (Solna, Stockholm) described strategies for inhibiting the expression of toxins produced by disease-causing microorganisms. Burman has studied the use of agents that selectively inhibit the production of toxin by *Clostridium difficile*, which is the etiological agent of pseudomembraneous colitis and a cause of antibiotic-associated colitis. The addition of cysteine, for example, to *ex vivo* colon models was found to downregulate toxin production. This work indicates that both acute and prophylactic application of anti-virulence drugs could be possible.

Burman's approach can be seen as an example of drugs being developed to alter the expression of genes in pathogens, rather than to just inhibit gene expression. This kind of drug could be widely applied to different circuits of gene expression that might possibly be common to many pathogens. Gary K. Schoolnik of Stanford University (Palo Alto, CA, USA) speculated on particular targets for controlling *Mycobacterium tuberculosis* by manipulating the bacterium in different ways during its latent and infectious periods. Schoolnik's group is using DNA microarray technology to pinpoint circuits that might serve as targets for such drugs. Thinking along this line is quintessential smart bullet: specificity at the gene level!

Restoring drug sensitivity

Agents that restore drug sensitivity to resistant pathogens could be used in combination with antibiotics to treat acute infections. Unless the agent was toxic in antibiotic-free environments, its prophylactic use would also not be expected to promote the evolution of horizontally mobile resistance-genes.

Robert E.W. Hancock of the University of British Columbia (Vancouver, Canada) discussed the efflux pump, OprM, of *Pseudomonas aeruginosa* as a target for this strategy. Efflux pumps are phylogenetically universal mechanisms for drug resistance, occurring in all cells from bacteria to those within human tumours[11]. Efflux pumps decrease the intracellular concentration of the chemotherapeutic agent by rapidly transporting it out of the cell. Dysfunctional OprM increases the sensitivity of *P. aeruginosa* to virtually all clinically relevant antimicrobial agents by up to 10^3-fold[16,17]. Agents that inhibited OprM could potentially be used to treat infections by restoring drug sensitivity to the pathogens. Again, such agents, used sparingly, would not be as likely to select resistant organisms. Because the function of OprM is important in many environments, loss-of-function mutants should be uncompetitive and, therefore, fail to propagate. However, inhibiting OprM function is not lethal except in the presence of antibiotics, thus the use of OprM inhibitors should not select *oprM* mutants with a different substrate specificity unless the inhibitors are overprescribed or prescribed as a prophylactic.

The horizontal evolution of genes

The nature of the selective forces that promote both virulence and the evolution of genes on the infectious elements of organisms that, in turn, cause us disease, should provide even more insights into the ways that infectious diseases might be managed. More research emphasis needs to be placed on horizontal gene transfer in its own right. It is a separate and powerful mechanism upon which evolution acts. It is already clear from laboratory evidence that competition between horizontally mobile genetic elements during horizontal transfer probably creates the most important constraints on their evolution[18]. This is in stark contrast to the casual supposition that the effects of the genes on the host organism are always the most important factors in their evolution[7]. We must move beyond the thinking that confines horizontal gene transfer to the mechanism by which organisms adapt to new environments.

I believe that casual perceptions of how microbes evolve[6], and even more so for how resistance evolves, are limiting the potential for the development of a new generation of therapies[11]. For too long we have relied upon casual, organism-centric views of evolution to guide our drug-development strategies. In part, this is probably a result of the common view that evolutionary theory is descriptive and retrospective, and of little immediate predictive and mechanistic value. However, that view is out of date.

There is increasing justification for focusing on evolutionary experiments to advance drug-discovery agendas. Ehrlich's 'magic bullet' concept appeared well before much was known about the physiology of microorganisms, when even less was known about their genetics and when essentially nothing was known about their evolution. The study of horizontal gene transfer is younger still. By this, I do not mean to neglect the important work on the biochemical mechanisms of transfer and intragenomic recombination, or the almost daily discovery and description of genes that were transferred horizontally[19]. However, as an independent field, in which the evolutionary forces shaping the elements that transfer are as seriously studied as the effects of the elements on the organisms they transfer to, there is comparatively little work[8,20]. Therefore, it is to be expected that the development of disease-treatment strategies has traditionally been better

informed by biochemistry and genetics than by evolutionary mechanism.

Evolutionary reactionism is what best characterizes our present drug-development approaches. From a sophisticated understanding of evolutionary mechanisms we might have hope of securing smart bullets: agents that might manipulate, rather than react, to evolution. Smart bullets would be consistent with legitimate alternative priorities in drug development. Unfortunately, new drugs of this type could be more expensive to produce and might have more limited therapeutic spectra. However, they could last longer than conventional antibiotics and might be the only way for medicine to keep pace with resistance.

Smart bullet technology will also require a commensurate advance in diagnostics technology. To apply smart therapeutics, the disease-causing agent and its relevant virulence determinants will have to be determined quickly, and from little material. PCR brought a revolution to diagnostics, but further advances are required for the full efficacy of smart bullets to be realized.

Conclusion

I believe there is, again, reason for optimism in treating infectious disease. Drug design informed by evolution rather than reaction to evolutionary consequences is our best current hope. It will take time for evolutionary theory to match the power of genome sequencing for the rapid identification of new potential targets, but the formal generation and testing of evolutionary hypotheses has begun to produce interesting insights. We must broaden our view of targets and reconsider our near exclusive use of antimicrobial strategies.

Acknowledgements

I am grateful to the organizers of the *2001 International Symposium on Progress in Drug Discovery and Development Sciences*, Bangalore, India, and to AstraZeneca Research Foundation India for sponsoring me to attend the conference. I thank Ø. Olsvik for advice on the manuscript.

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