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Phage therapy

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There is a renaissance of interest in the antimicrobial potential of phages as more pathogens become multiply antibiotic resistant. Phage therapy is not a new concept, and it is important to ask why it is not part of the current repertoire of western medicine despite the fact that it has been continuously and extensively used in Eastern Europe for almost a century. Answering this question successfully will, largely, determine whether phage therapy can gain the credibility needed to overcome the scientific, financial and regulatory hurdles facing its adoption in mainstream clinical practice. Despite a paucity of such information from human studies, pharmacokinetic data and clinical outcomes from animal studies are currently providing convincing evidence for the safety and efficacy of phage therapy.

Bacteriophages, more commonly referred to as phages, are viruses that infect bacteria. They were discovered in 1915 and attempts to evaluate their therapeutic potential began as early as 1919. The early history of phage therapy has been the subject of several recent reviews [1–3]. Essentially, there are three types of phages, obligately lytic, temperate and chronic. Obligately lytic phages, sometimes referred to as virulent, will kill their bacterial host, following infection, by lysing the infected cell to release progeny phages. Temperate phages may kill the host cell following infection but are also capable of forming a long-term stable relationship with the host. This relationship commonly involves the phage genome becoming integrated into the genome of the host as a prophage. A chronically infecting phage, typically a filamentous phage, can release progeny into the extracellular environment without killing its host, which can continue to grow and divide [4]. It is because of their ability to kill the infected bacterial cell that the obligately lytic phages have been used for phage therapy up till now and are currently thought to have real therapeutic potential. Attention, however, is also beginning to be paid to temperate and chronic phages. The fact that phages are self-replicating in the bacterial host is considered to offer a great advantage over antibiotics as phages will continue to amplify themselves at the site of infection as long as there are sensitive bacterial hosts to infect. Phages may also offer several other advantages over antibiotics. Phages typically are highly specific in terms of the bacterial species that they will infect and, indeed, commonly will only infect certain strains of any species. This host specificity of phages is both an advantage and disadvantage of phage therapy when compared to antibiotic treatment. Phages have a specific effect on the target bacterium and do not disrupt the normal microflora, but a precise identification of the target bacterium is required before an appropriate phage can be selected for therapy. Finally, problems with unwanted side effects and the development of resistance are likely to be much reduced in the case of phage therapy.

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Why is not phage therapy in current use in Western medicine?

The use of phage therapy has persisted without interruption in Eastern Europe, particularly in centres such as the Eliava Institute of Bacteriophage, Microbiology and Virology in Tbilisi, Georgia and the Institute of Immunology and Experimental Therapy in Wroclaw, Poland [2,5]. So why has phage therapy disappeared as an anti-infection strategy in Western medicine? To answer this question it is necessary to examine some historical aspects of the use of phage therapy in the West, which can be divided into four periods: 'early enthusiasm, crucial scepticism, abandonment, recent interest and reappraisal' [3]. Following the discovery of phages, which itself is a somewhat controversial topic [6], there was a rapid progression to the first human therapeutic use of phage to treat bacillary dysentery (for review see [3]). Commercialisation of

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phage therapy was rapid. For example, in the 1930s Eli Lilly produced at least seven phage products for human use, which were used to treat a range of conditions including abscesses, suppurating wounds and respiratory tract infections [2]. Three commercial anti-staphylococcal phage preparations produced by Eli Lilly, E.R. Squibb and Sons, and Swan-Myers (a division of Abbott Laboratories) were the subject of an investigation by Straub and Applebaum to establish whether they contained clinically useful phages [7], and it was concluded that they variously lacked a phage, contained a 'weak' phage or exhibited marked batch-to-batch variation. The American Medical Association's Council on Pharmacy and Chemistry report in 1934 on phage therapy ([8] as cited by [3]) was ambiguous regarding the efficacy of phage therapy and expressed concerns regarding the lack of understanding of the true nature of bacteriophages. The report also expressed concern 'that the lack of standardisation of phage preparations and the lack of criteria for purity and potency made it impossible to compare most of the studies that had been published' [3]. A potent illustration of the inadequate understanding of the biological nature of phages is provided by the addition of 'preservatives' such as phenol to phage preparations that would have rapidly inactivated any phages present. It was not until 1940 that the particulate nature of phages was clearly confirmed by electron microscopy [9] and the 'modern' study of phages was beginning, by which time the discovery of antibiotics was diverting research attention in the USA and elsewhere away from phage therapy. Ironically, it appears now in the case of some antibiotics that they can act synergistically with phages [10].

Reputable animal data

The resurgence of interest in phage therapy that we are currently experiencing is driven by the need for novel anti-infectives and is given some credibility by its continued use in Eastern Europe. This interest is, however, intellectually underpinned both by the enormous expansion of our understanding of phage biology, largely deriving from the pivotal role of phages such as λ in the development of molecular biology, and the positive results reported from a series of rigorous animal studies. One of the first of these rigorous studies was carried out by Dubos *et al*. in 1943 [11], and it examined the protective effect of phage against experimental *Shigella dysenteriae* infections in mice. The most influential studies were, however, carried out by Smith and Huggins on *E. coli* infections of mice and farm animals. Smith and Huggins [12] reported that a single intramuscular injection of a phage that recognised the K1 capsular antigen of the infecting *E. coli* strain was more effective than multiple intramuscular injections of various antibiotics (tetracycline, chloramphenicol, ampicillin and trimethoprim + sulphafurazole) in protecting mice against a potentially lethal intramuscular or intracerebral challenge. These results were confirmed recently and demonstrated to be quantitatively and qualitatively robust [13]. In 1983 Smith and Huggins [14] evaluated the efficacy of *E. coli* phages in the prophylaxis and therapy of neonatal enteritis in calves piglets and lambs and demonstrated a high degree of protection. The studies in calves were successfully extended in 1987 [15]. In 1991 it was shown that a phage, when inoculated into newly hatched chickens simultaneously with any of the three strains of *Salmonella enterica* serovar Typhimurium, produced a considerable reduction in mortality in the birds [16]. Since the early 1990s the number of animal studies has expanded rapidly and prophylactic and/or therapeutic benefits have been reported for a range of experimental infections including vancomycin-resistant *Enterococcus faecium* [17], *Clostridium difficile*-induced ileocecitis [18], *Pseudomonas aeruginosa* [19–21], *Staphylococcus aureus* [22–24], *Mycobacterium avium* [25], *E. coli* [26,27] including ESBL (Extended Spectrum Beta-Lactamase) strains [28] and *Vibrio vulnificus* [29].

Safety in humans

Phage interactions with animals in general and human beings in particular have been comprehensively reviewed [2,30], and there have been no reports of significant adverse reactions despite their long history of administration to humans. Studies by Ochs and his co-workers, for example Ochs *et al*. [31], described the use of phage to study immunodeficiencies in normal and patient populations where high concentrations of phage were administered intravenously without any toxic effects, despite the potent antigenic properties of this particular phage ΦX174. Given the abundance of phages in the environment, as well as in and on the human body, it is certain that they are presented to the immune system. For example, it has been reported that 11% of healthy controls and 23% of patients had antibodies against a *Staphylococcus* phage before its administration for therapeutic reasons [32]. Vaccines have been found to be contaminated with phages and their continued use was allowed by an executive order [33]. The veterinary product Staphage Lysate (SPL)® produced by Delmont Laboratories Inc. in the USA was, in fact, used in humans from the 1950s to 1990s and samples of SPL have been tested and found to contain 10^8–10^9 plaque-forming units (pfu) ml^{-1} [2]. As of August 18, 2006 the FDA announced that it had approved the use of the LMP-102 bacteriophage preparation, from Intralytix Incorporated (USA), made from six individually purified phages to be used on Ready-To-Eat meat and poultry products as an antimicrobial agent against *Listeria monocytogenes*. The product is reported to be effective in killing over 170 strains of *Listeria* and will be sprayed onto meat products. The FDA accepted that the product was safe when it considered various published literature articles on the use of phage in animal and human studies. A food safety phage company, EBI Food Safety located in Wageningen, The Netherlands, develops and markets natural bacteriophage products against dangerous food pathogens. This company has now achieved GRAS (Generally Recognised As Safe) status, for all Food Products, for its *Listeria* product LISTEX™ P100, by the USDA and FDA. This is confirmation that the FDA's view of phage is that they are safe for human use and opens the doors for phage commercialisation in human applications. The first phage safety test in human beings in the recent English literature described the administration, in their drinking water, of *E. coli* phage T4 to healthy adult volunteers and no adverse reactions were detectable [34].

Current status of phage product development

Phages are being commercialised in a number of areas (see Table 1 for the companies involved), and it is always important to consider the protection of an idea through filing Intellectual Property (IP) although this has not been easy as there have been so many examples of the use of phages in the past. Apart from human phage therapy, IP has been filed in the following areas: decontamination (farm yards, food produce and instruments), use of lysins or other phage enzymes, diagnostics, veterinary therapeutics, gene therapy delivery vehicles and vaccines. Lysins have enjoyed success in a variety of animal models to control Gram-positive pathogens [35]. IP has also been filed relating to a number of aspects of human phage therapy, and regulatory opinion is currently supportive of finding new approaches to overcome the

TABLE 1

A selection of company's active in the commercialisation of phage products.

Company	Primary location	Primary product area	Phage technology	Stage of development
BigDNA (http://www.bigdna.com/)	Edinburgh, UK	Bacteriophage DNA vaccination via phage encoded DNA delivered intravenously or orally	Bacteriophage DNA vaccines	R&D
Blaze Venture Technologies (http://www.blaze-vt.com/)	Hertfordshire, UK	Phage immobilisation technology, MRSA, licensing	Immobilisation onto solid supports	Licensing
JSC Biochimpharm (http://www.biochimpharm.ge/)	Tbilisi, Republic of Georgia	Various phage lysates are mixed and used for intestinal problems, for example, Dysentery, salmonellosis, dyspepsia, colitis and enterocolitis and for bacterial infections.	Whole phage	Phage tablet or liquid production facility
Biopharm L Limited (http://www.biopharm.ge/) that owns Advanced Biophage Technologies (http://advancedbiophagetechnologies.com/)	Tbilisi, Republic of Georgia	Products include Pyobacteriophage and Intesti-bacteriophage that are mixtures of phage lysates for bacterial intestinal and infection control—sold to pharmacies as Over The Counter drugs.	Whole Phage, patented and licensed.	Liquid and tablet phage products
BioControl (http://www.biocontrol-ltd.com/)	Southampton, UK	*Pseudomonas* infections of the ear	Whole Phage	Phase II trial completed
Biophage Pharma Inc. (http://www.biophagepharma.net/)	Montreal, Canada	Environmental therapies and diagnostics, phage products geared towards antibacterial resistance problems and as a weapon against bioterrorism	Whole phage	Research and development
EBI Food Safety (http://www.ebifoodsafety.com/)	Wageningen, Netherlands	Food Safety. A cocktail of phage against *Listeria*	Whole Phage	LISTEX P100^{TM}, product available
Gangagen (http://www.gangagen.com/)	Bangalore, India and Palo Alto, California, USA	*Staphylococcus aureus*	Whole Phage	Pre-clinical
Innophage (http://www.innophage.com/)	Porto, Portugal	Environment, Cosmetic and Medical bacteria infections	Unknown	Unknown
Intralytix (http://www.intralytix.com/)	Baltimore, USA	Food safety, *Listeria*	Whole Phage	FDA and EMEA approval on ready to eat meats and cheeses
Neurophage Pharmaceuticals (http://www.neurophage.com/)	Cambridge, MassachusettsUSA	Brain changes, for example Alzheimers	Unknown	Start up company
Novolytics (http://www.novolytics.co.uk)	Coventry, UK	Prevention and treatment of MRSA infection	Whole Phage	Pre-clinical
Omnilytics (http://www.omnilytics.com/)	Salt Lake City, Utah, USA	AgriPhage is a natural, safe, effective treatment that prevents and controls harmful bacteria on tomato and pepper plants	Unknown	Product available
Phage-Biotech (http://www.phage-biotech.com/)	Rehovot, Israel	Anti-*Pseudomonas* infectives	Whole Phage	R&D
Phico Therapeutics (http://www.phicotherapeutics.co.uk/)	Cambridge, UK	Anti MRSA products	Genetically Modified Organism used as a delivery vehicle	Pre-clinical
Phage International (http://www.phageinternational.com/)	San Ramon, California, USA; Trinidad, West Indies and Tbilisi, Republic of Georgia	Phage treatment centre	Whole Phage	Distributor
Special Phage Holdings Pty Ltd (http://www.specialphageservices.com.au/)	Brookvale, NSW, Australia	R&D	Whole phage	Prototype products, some entering clinical trials
Targanta Therapeutics (http://www.targanta.com/)	Cambridge, Massachusetts, USA	Antibiotics	Phage peptides	R&D
Viridax (http://www.viridax.com/)	Boca Raton, Florida, USA	*Staphylococcal aureus*—respiratory, systemic, topical, wound care	Whole Phage	Pre-clinical

problem of antibiotic resistance. It is, however, important to distinguish two approaches for which the regulatory issues will be significantly different. Currently, one approach adopted by PhicoTherapeutics (UK) involves the genetic modification of a phage to carry a gene encoding a protein that is generally toxic to bacteria. This has the advantage of being a platform technology (SASPject™) that is potentially applicable to any bacterium but suffers from the disadvantage that the phages do not replicate, and therefore, there is no amplification at the site of infection. Additionally, there may be substantial regulatory hurdles because of the GM nature of the phage being used. Other companies (e.g. Intralytix, BioControl, BioPhage Pharma and Novolytics) are employing naturally occurring obligately lytic phages or spontaneous mutants derived from them commonly as a cocktail of several phages aimed at infecting the large majority of the clinically significant strains of a particular pathogen. As there are no current phage therapy products in the market in the Western world, it is essential that the phage companies adhere to proper drug development strategies that give the field a proper chance of success. In the UK, BioControl have led the first UK trial of a phage product against *Pseudomonas* infections of the ear. After early success in ear infection in dogs (personal communication) BioControl have since completed the first Phasel/IIa trial in humans. There were no reported problems in getting approval from the UK regulators, the MHRA, and the company is now pursuing a phase III trial in the near future.

A number of phage companies have identified the large markets that are available for phage products against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Clostridium difficile*. Several of the companies listed in Table 1 state that they are working in this area, but only three, Novolytics Limited (UK), Phico Therapeutics (UK) and BioPhage Pharma Inc. (Canada) are anywhere near clinical trials. The approach that these companies are taking is based on prevention rather than cure. Nasal carriage of *Staphylococcus aureus* is now widely accepted as a risk factor for autoinfection with the same strain, and it is becoming increasingly commonplace that patients undergoing elective surgery are screened for nasal carriage of MRSA. The phage preventative approach would be to eradicate the nasal passage of MRSA before hospitalisation that should reduce the incidence of infection. This approach has fewer and lower regulatory hurdles than going directly for an intravenous therapeutic and will potentially get phage products to market far more quickly.

Conclusions

Historically, many of the limitations and failures of phage therapy can be attributed to a lack of understanding of phage biology and now seem naive. Current criticisms of phage therapy, however, largely focus on the quality of this historical evidence. It is clear that during the course of the next few years a variety of phage therapy products will undergo clinical trials, and rigorous assessments can be made regarding their safety and efficacy that should once and for all establish whether phage therapy is to have a place as an anti-infection strategy in Western medicine. In the meantime, further expansion of phage therapy as an experimental treatment in accord with current international standards (written informed consent of patients, approval by an institutional review board, and the necessary insurance policies—an approach implemented at the Wroclaw Institute in the past three years) could be considered in cases when antibiotics fail.

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