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BACTERIOPHAGE THERAPY – OLD TREATMENT, NEW FOCUS?

The control and management of infections and infectious diseases using antibiotics faces ever-increasing challenges from the development and spread of resistance to such compounds. This has become a major problem worldwide, and there are fears that the clinical management of many infectious diseases will become severely constrained. As a consequence a number of alternative antimicrobial strategies are currently being investigated. One approach to antibacterial therapy which has been used since the beginning of the twentieth century is the application of bacteria-specific lytic viruses or bacteriophages. The history, biology and early therapeutic use of bacteriophages can be found in a number of reviews and textbooks (Kutter & Sulakvelidze, 2005; Hanlon, 2007; Abedon, 2008) and this article will examine some of the recent developments and obstacles in the field of bacteriophage therapy.

Early development of bacteriophage therapy

Much of the pioneering work with bacteriophages and bacteriophage therapy was inconclusive and the therapeutic outcome sometimes worse than the presenting infection, leading to mistrust and scepticism. The 'Bacteriophage Inquiry' in India in 1927 and an American Medical Association review in 1931 failed to produce conclusive evidence as to efficacy of the therapy. The development of antibiotics and antimicrobial chemotherapy at around the same time halted further serious investigation, and by the end of the Second World War, bacteriophage therapy was seen as a marginal curiosity.

Research on bacteriophages did not stop with the advent of antibiotics but continued in countries in the former Soviet Republics, where antibiotics were inaccessible. Advances in knowledge, techniques and application increased so that a substantial body of evidence accumulated, and active programmes were initiated to develop and distribute bacteriophage therapeutics throughout the Soviet bloc. The Eliava Institute in Georgia became the premier bacteriophage research establishment, producing a wide range of preparations containing bacteriophage and 'cocktails' of specific bacteriophages. Incidentally, Félix d'Hérelle (who announced his discovery of bacteriophages in 1917, independently of Frederick Twort who first described their lytic behaviour in 1915) introduced bacteriophages to George Eliava in 1926 on a visit to the then Tbilisi Institute of Bacteriology.

Resurgence of interest in the West

After the collapse of the Soviet Union in 1989, bacteriophage therapy came to the attention of the West once again, this time with supporting evidence of therapeutic efficacy observed in Eastern Europe. Unfortunately, language difficulties and the lack of detailed documentation and double-blind controls in many of the trials, meant that the body of work as a whole was not regarded seriously, and there was insufficient evidence for its acceptance and approval for use as a therapeutic option.

However, the spectre of increasing levels of multi-antibiotic resistance focused minds once again on bacteriophages as therapeutic tools. Basic research into the biology and ecology of bacteriophages, together with advances in molecular biological techniques and purification methods, enabled the development of well characterised, specifically targeted and highly purified bacteriophage mixtures for therapeutic use, and scientists and clinicians in Europe and America began the first clinical trials.

For example, a cocktail of eight bacteriophages (five against *Pseudomonas aeruginosa*, two against *Staphylococcus aureus* and one against *E. coli*) was used to treat infected leg ulcers in Texas, USA (Marza *et al*., 2006). The bacteriophages were supplied by Intralytix, a US company founded by a former Georgian microbiologist. Following the trial, the Southwest Regional Wound Care Centre in Texas used bacteriophages in conjunction with other methods, to treat antibiotic-resistant infections (www.woundcarecenter.net). The use of bacteriophage T4 against *E. coli* in cases of diarrhoea is being assessed in Bangladesh by Nestlé, the Swiss multinational food corporation. This follows safety testing on human volunteers (Bruttin & Brüssow, 2005). Phase 2a clinical trials were conducted in 2007 at the Royal National Throat, Nose and Ear Hospital in London, on the treatment of chronic inner ear infections caused by *Pseudomonas aeruginosa* using a cocktail of six bacteriophages produced by the UK start-up company Biocontrol. Very positive results have been reported from the trial in terms of clinical and bacteriological efficiency and safety concerns. The therapeutic cocktail is currently being examined in phase three trials. Administration of the bacteriophage cocktail was via an aerosol delivery system which was granted a patent by the European Patent Office in 2008. A similar concept involving the nebulisation of bacteriophages of *Burkholderia cepacia* Complex to treat cystic fibrosis has recently been described (Golshahi *et al*., 2008).

As well as clinical trials, basic research has been ongoing. At the 17th Biennial International Evergreen Phage Biology Meeting held in Olympia USA in August 2007 bacteriophage therapy sessions covered a range of infections and bacteria such as Otitis Media and *Pseudomonas aeruginosa*, urinary tract infections (UTIs) and *Klebsiella* species, *Burkholderia*, Group A *Streptococcus,* and *Bacillus anthracis* infections (http:// academic.evergreen.edu/projects/phage/). Despite all the interest and debate, few clinical evaluations of bacteriophage therapy have been published to date in the West. A possible reason for this lack of advance may be that the data available are at a very early stage with few meaningful and significant results, thus making it difficult to attract funding. Secondly, bacteriophage therapy is somewhat of a grey area to most regulatory agencies and consequently questions of medical ethics and procedural pathways abound. Within existing frameworks it is virtually impossible to start clinical investigations to generate the data required to demonstrate the safety and efficacy of bacteriophage-based therapeutics. It should also be noted that in the former Soviet Republics, the vast majority of bacteriophage preparations were designed for use as non-systemic medications i.e. lavage, sprays, ointments and dressings. Several preparations were for oral or rectal (suppository) delivery, and the few injectable preparations were for intramuscular or intraperitoneal administration. Thus nearly all the work from Eastern Europe involved topical and localised applications, for example 'PhageBioderm' a biodegradable material impregnated with bacteriophage, designed for the treatment of skin infections, and 'PhageBiodent' for periodontal and gingival applications.

Safety concerns

A number of companies focussing on the development of bacteriophage therapy were formed during the 1990s to exploit the niche for intravenous antibacterial therapeutics. However the combination of clinical trial requirements, problems with unwanted immunological reactions, and intellectual property rights issues led many of these companies to turn to veterinary and agricultural applications, which were perceived as less regulatorily tricky areas. Such a change in direction resulted in the US Food and Drug Administration approving a product developed by Intralytix in 2006. The product contained bacteriophages active against *Listeria monocytogenes* and was designed as a disinfectant spray for packaged meats. Subsequently, other products from companies such as Omnilytics and EBI Food Safety have been approved by the US FDA and given approval under the 'generally regarded as safe' (GRAS) system. The approval of these products shows recognition that bacteriophages are considered safe for use on food destined for human consumption. This is a step towards acceptance of bacteriophage therapy in general. The

ubiquitous nature of bacteriophages, estimated to be the most numerous organisms on the planet (Abedon, 2008) means that humans are exposed to them from birth, and have been in symbiotic relationship with them for thousands of years. In fact it is impossible not to ingest bacteriophages since they form part of the natural flora of the human oral cavity and gastrointestinal tract, and are present in municipal drinking water and various food items.

There were also concerns regarding the safety of intravenous bacteriophage preparations, a problem not readily encountered in Eastern Europe since there was a preference for topical applications in those countries. Bacteriophages have the potential to elicit strong immune responses mainly due to their protein content, and as a consequence the system can clear the bacteriophages from the body rapidly. The interaction between bacteriophages and antibodies is of concern because if the bacteriophage elicits an antibody mediated response, further treatment with that particular bacteriophage would be negated. Another concern over the use of intravenous bacteriophage preparations is the chance of the patient developing the Jarisch-Herxheimer reaction - systemic shock resulting from the sudden release of polysaccharide endotoxins into the bloodstream during the lysis of susceptible bacteria. The problem of lytic toxicity has been investigated specifically in a veterinary trial of active bacteriophage therapy, and no significant adverse reactions or effects were noted (Soothill, 2004).

The wealth of data from Eastern Europe suggests that bacteriophage therapy is safe (Sulakvelidze, 2005), and experience at clinics where bacteriophage therapy is provided indicates that the treatment is effective and without negative side-effects. One of the largest bodies of published work in English on bacteriophage therapy comes from the Hirszfeld Institute of Immunology and Experimental Therapy in Wroclaw, Poland. Their experience over the past thirty years in treating nearly 2000 patients suffering from a variety of often life-threatening infections was very positive, with an overall rate of success of 60% to 90% and no reports of serious adverse reactions (Stone, 2002).

Novel approaches to bacteriophage therapy

Until recently, bacteriophage therapies have been based on complete virus particle preparations or purified bacteriophage lytic enzymes such as lysins and holins (Fischetti, 2005). Novel developments include ‘phage display, where an antibacterial peptide or protein is displayed on the surface of a genetically modified bacteriophage. The bacteriophage is designed not to lyse but rather to deliver the attached antibacterial to the target bacteria. (Westwater *et al.* 2003). Bacteriophages have also been used as potential vehicles for the delivery of vaccines. The bacteriophages can carry antigens on their surface or deliver a DNA extension cassette that has been engineered into the bacteriophage genome (Clark & March, 2004). The UK company Phico Therapeutics Ltd. are developing engineered ‘improved’ bacteriophages for intra-nasal decolonisation of MRSA and for oral therapy against *Cl. difficile* associated disease (http://www.phicotherapeutics.co.uk/).

The major problem with these approaches is the approval of genetically engineered agents, added to the fact that the bacteriophages themselves are not categorised as therapeutic modalities, thus proving a real challenge to pharmaceutical companies wishing to pursue this route. Two of the major bacteriophage companies, Intralytix and Biocontrol, are only working with ‘natural’ or unmodified bacteriophages for these very reasons.

Obstacles to the acceptance of bacteriophage therapy

Despite the accumulating biological, molecular and clinical evidence supporting the use of bacteriophage therapy, there are a number of hurdles to be overcome before it is accepted and considered a therapeutic option. These problems can be regarded as biological, proteomic and

regulatory. For example, little is known about bacteriophage interactions in the gut and other anaerobic environments, and some work has shown that T-even bacteriophage display oxygen-dependent growth on their host strain and inhibition of lysis under anaerobiosis. It is also thought that bile salts and gut carbohydrates may sequester the bivalent metal ions needed by bacteriophages for adsorption and replication (Chibani-Chennoufi *et al.* 2004).

The phenomenon of bacteriophage mutation leading to resistance has also been cited as a concern, and resistance to the therapeutic bacteriophage has been noted in some animal studies. However the use of bacteriophage cocktails has the potential to minimise or prevent the development of resistance.

The conversion of a lytic bacteriophage into a lysogenic lifecycle could be a major problem if the bacteriophage genome integrates with that of its host. Subsequent reactivation could result in the transfer of bacterial virulence factors and the bacteriophage mediated transfer of virulent bacterial genes into other bacteria. In order to avoid this, care must be taken to ensure that the potential therapeutic bacteriophage is predominantly or wholly lytic, and does not carry any toxic genes or housekeeping genes for initiating lysogeny. There are little data on the genomes and proteomes of lytic bacteriophages, so questions of lysogenic, toxic or virulent genes remain unanswered at present.

From a regulatory point of view, bacteriophages are rather ambiguous, and the fact that they are self-replicating biological entities is another complication. To meet current regulatory approval, such a product must comprise of a highly purified, characterised, and validated bacteriophage or mixture of bacteriophages, together with optimised administration protocols, supported by properly controlled efficacy and safety studies. There are no specific frameworks for bacteriophage therapy in the current Medicinal Product Regulation (EC, 2001), so short-term borderline solutions under the responsibility of a medical ethics committee or under the umbrella of the Declaration of Helsinki are possible options. It has been proposed (Verbeken *et al.* 2007) that a long-term solution would be the creation of a specific section for bacteriophage therapy under the Advanced Therapy Medicinal Product Regulation (EC, 2003).

The development of bacteriophage therapeutics is also hindered by issues of intellectual property rights. Bacteriophages have been used as therapeutic agents for almost a century and are thus unpatentable. As a consequence, few pharmaceutical companies would be willing to invest the sums of money required to develop a product for bacteriophage therapy unless their results are ‘protected’ by an international patent.

Another potential obstacle for the clinical application of bacteriophages is the perceived ‘fear’ of viruses. Viruses are seen by many members of the public as ‘like bacteria but more dangerous’ or as ‘enemies of life’, and the idea of injecting them into patients or spraying them onto food raises real if unwarranted concerns.

Is there a future for bacteriophage therapy?

If the necessary regulatory and ethical hurdles are overcome and bacteriophages become accepted as therapeutic agents, this could stimulate the development of new approaches and methodologies. It is possible that initially agricultural, veterinary, food hygiene and food safety applications will predominate until sufficient data from clinical and safety trials are available. New

pharmacokinetic data, advances in biocompatibility studies and a greater understanding of bacteriophage-bacteria interactions will enable novel targets to be identified and the efficacy of bacteriophage-based therapies to be improved. It is also possible that bacteriophage therapy will be approached like other biological control methods used in agriculture, with the aim to reduce bacterial infections and spread of diseases using a range of modalities and therapeutic agents, as part of an 'integrated pathogen management' plan.

At the start of the twentieth century bacteriophage therapy was seen as the answer to bacterial infections but initial therapeutic problems and the advent of antibiotics pushed it into the background. Now at the start of the twenty-first century with the ever increasing problem of antibiotic resistance, we have the wealth of clinical experience from colleagues from the former Soviet Republics and the necessary biological knowledge and tools to re-evaluate the therapeutic potential of these 'bacteria eaters'.

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