editorial

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Biologics: a new approach needed?

Growing importance of biologics

Biologics are growing in value and importance as a proportion of new drug approaches. Biologics (biological macromolecules: polysaccharides, polynucleotides (DNA and RNA) and polypeptides (proteins), including monoclonal antibodies) are forecast to make at least c.55% of the increase in prescription pharmaceutical sales from 2007 to 2012 [1].

The reason for this growing importance is that small molecules, which currently account for 81.4% of total pharmaceutical revenues, are expected to see slow sales growth over the next few years. This is, in part, because the blockbuster drugs expected to go off patent and face heavy generic competition are almost exclusively small molecules [1].

To date, biologics tend to address diseases with a large unmet medical need, which lack alternative therapies. This means there is a high commercial demand for these products and the potential for blockbuster-level annual revenues.

Overview of current biologics pipeline

Although biologics look increasingly promising, the majority of molecules in the clinic or in development are monoclonal antibodies or alternative binding scaffolds aimed at extracellular targets. This has meant that the industry often stereotypes biologics as proteins that affect biological activity by binding to targets.

There are two important reasons why the biologics industry has developed in this way. The first is historical. The major discoveries that led to today's 'large molecule' protein-based therapeutics occurred in the 1970s with the development of recombinant DNA technology and work by scientists including Kohler and Milstein that made monoclonal antibodies possible [2]. The types of biologics now entering the market are the direct result of several decades spent commercialising this research.

The second reason is practical. Biologics are large, charged molecules, such as proteins and nucleic acids, which do not readily cross cell membranes. Targeting the correct cell type and delivering the molecule into the cell is a major challenge – it is simpler to develop a biological molecule that acts outside a cell. Also, the intracellular environment is reducing and not well suited to biologic protein scaffolds that involve essential di-sulphide bridges to maintain functional tertiary structures.

The molecules that have been designed to successfully enter and function within the cell, such as intrabodies, are again binding scaffolds that do not display enzymatic activity. This limits the range of therapeutic effects that they can have. The lack of catalytic activity also means that large numbers of the molecule have to be delivered into the cell to achieve a meaningful functional effect. The delivered biologics are also vulnerable to degradation pathways evolved by cells to remove foreign biological molecules.

A challenge for developers of biologics is, therefore, developing a therapeutic molecule that targets intracellular mechanisms and has potent and specific effects on intracellular pathways and processes. Many bacterial toxins have evolved to do just that, often being highly specific enzymes that target specific intracellular pathways within the host cell. As such, they offer an exciting opportunity to develop new and effective therapeutics.

Despite the advantages offered by bacterial toxins as intracellularly active biologics, there are only two pioneering examples of protein therapeutics based on bacterial toxins. These are the successful clinical use of engineered botulinum neurotoxin and diphtheria toxin. There are other bacterial toxins that may be used as the basis of biologics in the future, but these remain the subject of basic research or speculation.

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This failure to use tools provided by microbial evolution to develop effective therapeutic proteins has limited the clinical needs that can be met by biologics to date.

Biologics based on cytolethal bacterial toxins

Several bacterial toxins, including diphtheria toxin and pseudomonas toxin kill cells by halting protein synthesis through specific enzymatic modification of the protein synthesis machinery. Ligand Pharmaceuticals Inc.'s Ontak™ (Denileukin diftitox) is an engineered cytolethal bacterial toxin based on diphtheria toxin. The drug is designed to treat Cutaneous T-cell Lymphoma (CTCL), a type of non-Hodgkin lymphoma that primarily affects the skin and is caused by the uncontrolled growth of T cells expressing the CD25 component of the Interleukin-2 (IL-2) receptor.

Ontak™ is a fusion protein consisting of an enzymatically active fragment of diphtheria toxin genetically fused to IL-2. Ontak™ uses IL-2 to target and bind to IL-2 receptors on the surface of malignant T cells. The enzymatically active fragment of diphtheria toxin then enters and kills these T cells [3].

Ontak™ is a lymphoma drug, but cytolethal toxins like diphtheria and ricin [4], could be engineered to treat other types of cancer and numerous other diseases involving inappropriate cell proliferation. The use of ricin to treat cancer, however, remains a matter for speculation [4].

Cytolethal toxins could also be potentially used in situations where cells are not dividing excessively, but are causing a disorder and can be killed without harming the patient. One such line of research is the use of saporin, a ribosome inactivating protein (RIP) from soapwort seeds, to kill spinal cord cells involved in chronic pain.

Research by Mantyh, Wiley and others has focused on attaching a peptide substance P to saporin. This substance delivers the saporin into superficial neurokinin 1 receptor (NK1r)-bearing cells in the spinal dorsal horn where it behaves as a cytolethal toxin [5]. P–saporin reverse chronic pain in rats [6]. Although saporin is not a bacterial toxin, this work demonstrates the therapeutic potential of targeting cytolethal toxins to specific cell types involved in a disease.

Designer proteins based on botulinum neurotoxin

Even more exciting than the cytolethal toxins as the basis for novel biologics platform are the many non-cytolethal bacterial toxins that have evolved to modulate the function of host cells by the enzymatic modification of specific intracellular signalling pathways.

Botulinum neurotoxin is routinely used in cosmetic and therapeutic treatment for relaxing and paralysing muscles. It generates this effect by blocking the release of acetylcholine, a neurotransmitter released by motor nerves to stimulate muscle contraction. For acetylcholine secretion to occur, a synaptic vesicle containing acetylcholine must fuse with the nerve cell surface. A complex formed of soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins mediates this process. Botulinum neurotoxin prevents this complex forming by cleaving one of the SNARE proteins [7].

The SNARE complex, however, is not just involved in acetylcholine release, nor is it restricted to nerve cells. It is a universal mechanism underpinning secretion from all cell types. The reason botulinum neurotoxin is not able to affect secretions from other cell types is because it has evolved with a highly targeted delivery mechanism that specifically binds to nerve cells.

Botulinum neurotoxin has been successfully engineered to alter its targeting and delivery mechanism so that it inhibits secretions in different types of cell. Since the engineered proteins, produced by expression from novel recombinant genes, can be designed to have no motor nerve binding, they are not neurotoxic. In addition, they, like botulinum neurotoxin, have a long period of action – up to several months, which makes them ideal for managing long-term, chronic diseases and conditions [8].

Given the ubiquitous involvement of SNARE proteins in secretions from all cell types, a novel biologics platform based on botulinum neurotoxin will potentially treat any disease involving secretion. Some potential applications include tackling chronic pain, inflammatory and endocrine disease. Current treatments for these conditions are often expensive, have major side effects and require regular medication.

Proteins based on botulinum neurotoxin have been designed to target mast cells or macrophages to inhibit the release of inflammatory mediators, such as histamine and cytokines. Such proteins would be useful in the treatment of inflammatory diseases. An advantage of such an approach is that the release of all inflammatory mediators from the target cell can be inhibited, thereby giving a broader anti-inflammatory profile than can be achieved by inhibiting the activity of a single mediator, as with current monoclonal antibody approaches such as anti-TNFα.

Proteins can be designed to target C fibres, the peripheral nerves that transmit pain signals, to inhibit the range of neurotransmitters released by these nerves. As with inflammation, this again offers a more effective therapeutic approach to reducing pain than drugs that only inhibit the activity of a single neurotransmitter, such as receptor antagonists or monoclonal antibodies.

Designer proteins based upon botulinum neurotoxin could also treat disorders caused by the inappropriate or over-release of hormone or growth factor secretion from pituitary and other endocrine cells. These include diseases caused by endocrine tumours like acromegaly and Cushing's Syndrome. Inhibiting hormone and growth factor secretion could also help treat certain cancers.

Potential therapeutic use of other toxins

There has been research and speculation into the potential therapeutic use of many other bacterial toxins. These include certain C3 exoenzymes produced by some strains of *Clostridium botulinum*. These exoenzymes are known to inactivate Rho GTPases in target cells, which are involved in numerous cellular processes, including cell-cycle progression, cell transformation, chemical transport and programmed cell death [9].

Another tool that could be stolen from bacteria are type III and IV secretion injectisomes and the toxins that they introduce into the cell. These injectisomes form a protein structure on the bacteria that it uses like a hypodermic syringe to inject a payload of proteins through cell membranes [10]. These payload proteins represent a diverse set of intracellular toxins evolved to affect numerous specific metabolic pathways and signal transduction mechanisms within the target cell. They, therefore, represent

another category of bacterial effector protein that could provide the basis for therapeutic biologic development.

Conclusion

It is obvious that the future potential of suites of drugs based on modified bacterial exotoxins or injectisomes effector proteins is enormous. Such engineered exoenzymes could affect many processes in cells. Although biologic platforms based on some of these concepts may be decades away, there are already biologics based on diphtheria toxin and botulinum neurotoxin in clinical use. Engineering bacterial toxins, therefore, opens up an opportunity to develop novel biologics platform that significantly expand the therapeutic applications offered by current biologics.

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