Institution: University of East Anglia

Unit of Assessment: 3A - Allied Health Professions, Dentistry, Nursing and Pharmacy: Pharmacy

Title of case study:

Targeting inflammation by keeping Keap1 away

1. Summary of the impact

Chronic, debilitating diseases such as arthritis, chronic obstructive pulmonary disease (COPD) and inflammatory bowel disease (IBD) could potentially be treated by damping down the underlying inflammation and therefore improving the quality of life of sufferers. Nrf2 is a protein that prevents inflammation when activated and many researchers have sought to manipulate its activity as a potential therapeutic strategy. However, this has had little success, due to a lack of suitable biochemical tools. We describe here the Nrf2-activating peptide TAT14, which was developed in Pharmacy and is now being marketed by biotech companies to study this important pathway.

2. Underpinning research

While inflammation is an essential response to stress, when its levels are abnormal or mistimed, this is linked to chronic diseases such as arthritis and COPD as well as contributing to the progression of others such as cancer and atherosclerosis. Consequently, the search for novel anti-inflammatory drugs with improved efficacy and reduced toxicity remains a priority for the pharmaceutical industry. One target that has recently attracted attention is the interaction between the cellular proteins Nrf2 (nuclear factor erythroid-derived 2) and Keap1 (Kelch-like ECH-associated protein 1). Under normal conditions, the binding of Keap1 to Nrf2 serves as a signal that marks Nrf2 for degradation, thus keeping the level of Nrf2 low in cells. During inflammation, the binding between Nrf2 and Keap1 is abolished. Free Nrf2 then binds to DNA and promotes the expression of protective anti-inflammatory genes. It therefore followed that blocking the Nrf2/Keap1 binding would increase Nrf2 levels and have an anti-inflammatory effect.

Maria O'Connell (Senior Lecturer, 2006-current) researched the potential involvement of Nrf2 in bacterial sepsis. Her 2008 paper was the first to show that Nrf2 induces anti-inflammatory genes in an *in vitro* model for sepsis, in which human monocytes were treated with lipopolysaccharide [1]. In follow-up studies, she set out to disrupt the Nrf2/Keap1 binding, seeing this as a potential mechanism for reducing inflammation. A number of small molecules were already known to have the ability to irreversibly modify Keap1 and block its binding to Nrf2. One of them, a modified plant natural product methyl bardoxolone (CDDO-Me), reached a Phase III clinical trial for the treatment of chronic kidney disease but this was halted due to toxicity. There are concerns that this and related molecules may non-specifically react with other cellular components *in vivo* and cause unwanted side effects.

O'Connell then decided to investigate the feasibility of reversible Keap1 binders that would not suffer from this disadvantage and initiated collaboration with **Mark Searcey** (Professor of Medicinal Chemistry, 2006-present). Exploiting the X-ray structure of the Nrf2/Keap1 complex, they hypothesised that a peptide sequence derived from the region of Nrf2 that binds to Keap1 might act as a surrogate for the full protein. This would then block Keap1 and free up Nrf2 to exert its anti-inflammatory effects. Working with two PhD students, **Richard Steel** (2010-2013) and **Jonathan Cowan** (2010-2013), they investigated three Nrf2-derived peptides of 10, 14 and 16 amino acids in length. Of these, the two longer peptides were shown to bind to Keap1 with high affinity in a reversible manner. Although the first objective of reversibly disrupting Nrf2/Keap1 binding was achieved in a test tube, such peptides have poor cell permeability. The next step was to conjugate the peptides to the membrane-penetrating TAT sequence derived from the human immunodeficiency virus (HIV). The 14 amino acid Nrf2 peptide conjugated to TAT (TAT14) not only bound Keap1 *in vitro* but also had good cell uptake [2]. In human monocyte cells, TAT14 significantly activated the expression of heme oxygenase-1, an anti-inflammatory gene downstream of Nrf2, at concentrations as low as 37.5 μM. The peptide also reduced the

expression of the pro-inflammatory cytokine TNF (tumour necrosis factor) in the lipopolysaccharide induced sepsis model.

3. References to the research

(UEA authors in bold)

Publications

1. **SA Rushworth**, **DJ MacEwan**, **MA O'Connell** (2008) Lipopolysaccharide-induced expression of NAD(P)H:quinone oxidoreductase 1 and heme oxygenase-1 protects against excessive inflammatory responses in human monocytes. (*58 citations*)
   Journal of Immunology **181**:6730-6737
   http://www.jimmunol.org/content/181/10/6730
2. **R Steel**, **J Cowan**, **E Payerne**, **MA O'Connell**, **M Searcey** (2012) Anti-inflammatory effect of a cell-penetrating peptide targeting the Nrf2/Keap1 interaction. (*5 citations*)
   ACS Medicinal Chemistry Letters **3**:407-410
   doi: 10.1021/ml3000041g

Research Funding

Targeting the Nrf2/Keap1 interaction: Studentship: Richard Steel, EPSRC Doctoral Training Account, 2010-2013

Targeting the Nrf2/Keap1 interaction: Studentship: Jonathan Cowan, UEA Dean's Studentship, 2010-2013

4. Details of the impact

Research reference 2 above led to immediate interest by the pharmaceutical industry. A large body of *in vitro* and *in vivo* evidence supports the hypothesis that disrupting the Nrf2/Keap1 interaction is a valid approach for anti-inflammatory therapy. Traditionally, the disruption of such protein-protein interactions has been highly challenging for the pharmaceutical industry. Nevertheless, small molecules had been found that bind to Keap1 and prevent its interaction with Nrf2. Although one of these (bardoxolone) has entered clinical trials, it attaches to Keap1 covalently, with the potential for undesired side effects by nonspecific binding. It would be preferable to achieve Keap1 blockage with a reversible competitive ligand, and work by O'Connell and Searcey was the first to show that the Nrf2/Keap1 interaction can be blocked in cells by a reversible ligand, provided that the molecule has high cell permeability. The results provide a valuable proof of concept that reversible Nrf2/Keap1 disruption has the same anti-inflammatory effects in cells as the earlier irreversible approach. This discovery lays the foundation for the discovery of second generation reversible ligands with improved drug-like properties over the original peptides.

Searcey was contacted by *Novartis*, who requested samples of the peptide TAT14 for their research. To quote [redacted] at *Novartis UK*:

[redacted]

(Corroborative source **A**)

Later, *Novartis* had a contract company make the peptide as the amount needed was too large for the Searcey group to synthesise in-house. Independently, *AstraZeneca* showed interest in the activity of TAT14 and carried out studies in their own laboratories, including Surface Plasmon Resonance (SPR) and other techniques to quantify the binding between the peptide and Keap1.

Following these expressions of interest from the pharmaceutical industry, TAT14 is already available in the catalogues of two pharmaceutical/fine chemical companies. In 2013, Searcey was approached by the biotech company *Tocris Bioscience* to discuss the sale of the TAT14 peptide as a chemical biology tool. *Tocris* describes the utility of TAT14 as follows:

"Currently, there are very few pharmacologically active and commercially available tools for studying this (Nrf2) transcription factor in cell culture. Nrf2 activation tends to be an ancillary activity for most of the small molecules *Tocris* sells for this target (e.g. curcumin, andrographolide, methyl fumarates). Therefore, a peptide selectively targeting Nrf2, particularly one with proven cellular activity should prove to be an important tool in the arsenal for cell biologists studying cellular stress mediated through the Nrf2/Keap1 pathway."

(Corroborative source **B**).

Tocris made TAT14 available for sale in March 2013 and cite reference 2 above on their web page (Corroborative source **C**). Within a few months, they had sold [redacted] TAT14. [redacted] the commercial potential of TAT14 will only be known after a full year on the market. Meanwhile, the American biotech company, *EMD Millipore Chemicals* (also known as *Calbiochem*), has introduced TAT14 for sale (Corroborative source **D**).

Although this research was only published in 2012, the commercialisation by two companies less than a year later highlights the utility of TAT14 for inflammation research and drug discovery.

5. Sources to corroborate the impact

A. Corroborative letter from [redacted], *Novartis* Horsham Research Centre, UK, held on file at UEA.

B. Corroborative letter from [redacted], *Tocris Bioscience*, UK, held on file at UEA.

C. *Tocris Bioscience* catalogue, webpage for TAT 14 peptide: http://www.tocris.com/dispprod.php?ItemId=372041#.Uj4mboakpCg

D. *EMD Millipore Chemicals* catalogue, webpage for TAT-14 peptide: http://www.millipore.com/catalogue/item/492042-10mg