BIOPHARMACEUTICALS

A suggestion of storms ahead

Recombinant human erythropoietin (epoetin) is one of the success stories of modern medicine. Erythropoietin is essential for the production of red blood cells (erythrocytes), and epoetin is used in the treatment of patients in whom production of the hormone erythropoietin is impaired, such as those with chronic renal failure. Epoetin is one of the world's best-selling drugs, with over US $2.5bn sales recorded each year. Its selectivity for receptors that are expressed only on erythrocyte progenitor cells gives the drug an enviable degree of specificity, and its therapeutic index is among the highest known. The report in a recent issue of the *New England Journal of Medicine* of a collection of the first serious adverse events to be associated with the medically directed use of epoetin is therefore a surprising cloud to appear on the horizon.

Epoetin has had an almost blameless record, with the only substantial adverse effects being reported so far in athletes who had taken the drug illicitly, in the hope of increasing their red-cell count — so-called 'blood doping'. The consequent rise in blood viscosity has led to several cases of fatal thromboembolism. But Casadevall *et al.* now describe 13 patients receiving epoetin as standard treatment for chronic renal failure who developed severe anaemia, which could be treated only by blood transfusion. In all cases, this anaemia was caused by the production of antibodies to erythropoietin, which neutralize both epoetin and any remaining endogenous erythropoietin.

What drives the immune response in this subgroup of patients is unclear. Interestingly, these 13 cases — plus another 30 or so incidences of anaemia that have arisen recently — have almost all occurred in Europe since 1998, and might possibly be the result of some change to the product formulation. Although the numbers affected might seem insignificant when compared with the 3 million patients who are treated with epoetin each year, the relatively sudden appearance of a serious adverse effect for a tried-and-tested product reminds us that much remains to be discovered about the immunogenicity of biopharmaceuticals.

Adam Smith

References and links

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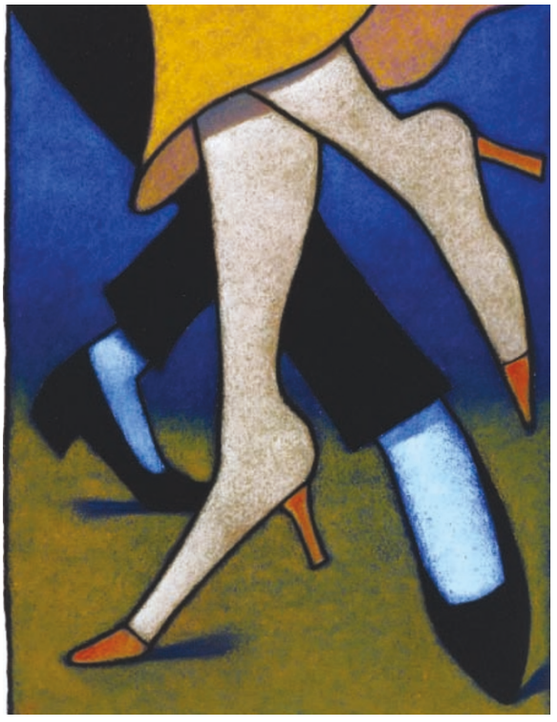
COMBINATORIAL CHEMISTRY

Clicking into place



In the fairy tale Cinderella, the prince has to go through the entire kingdom, trying to find his princess by testing people's feet to see if they fit a glass shoe. Time consuming, but not too bad compared with conventional searches for the 'feet' in drug discovery — that is, the drugs — which have often been performed without any knowledge of the protein 'shoe'. To stretch an analogy, structure-based drug design addresses this issue to some extent by using knowledge of the shoe to preassemble feet more likely to fit, but better still, what if the shoe itself could be used to make the ideal foot? This is the idea behind *in situ* 'click chemistry', a strategy developed by Barry Sharpless and colleagues, which could be thought of as testing a range of foot parts designed to link together in the shoe only if they can form a foot that fits perfectly. The first example of the application of this technique to the discovery of a femtomolar inhibitor of acetylcholinesterase (AChE) is described in a recent issue of *Angewandte Chemie*.

Click chemistry uses chemical building blocks containing functional groups that are thermodynamically 'spring-loaded' to react only with appropriate complementary functional groups in other blocks. The present study exploited the Huisgen 1,3-dipolar cycloaddition, which links azides and acetylenes — functional groups that are generally compatible with enzymes under physiological conditions, and that can easily be incorporated into a wide range of building blocks. The Huisgen cycloaddition can be carried out in water, and — ideally for the current purpose — the reaction depends on the proximity and appropriate alignment of the two components.

AChE, a target of drugs for Alzheimer's disease, was used as a template for the assembly of the building blocks — in this case, small molecules known to bind with affinities in the micromolar to nanomolar range to either the active site or an adjacent site of AchE. These were linked to azide or acetylene groups by alkyl chains of varying lengths. Each of a possible 49 pairs of small molecules was incubated with AChE, and subsequent examination of the reaction mixtures by mass spectrometry indicated that only one azide–acetylene pair, TZ2–PA6, had combined. No such reaction occurs in the absence of enzyme, and blocking the active site inhibited the formation of TZ2–PA6, showing that the active site is acting as the template.

TZ2–PA6 is the strongest non-covalent inhibitor of AchE discovered so far by two orders of magnitude. It seems likely that broader applicability of the *in situ* click chemistry approach will be the subject of much future research.

Peter Kirkpatrick

References and links

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