mate concentrations *in vivo* in the MCAO model. Measurements using microdialysis probes showed an 80% reduction in extracellular glutamate concentration in the caudate nucleus of the brain and a complete prevention of ischaemia-
induced glutamate buildup in the parietal cortex[4]. By contrast, there was no significant effect on glutamate levels in normal, non-ischaemic rats. This apparent selectivity could explain the relative lack of side effects seen with 2-PMPA.

'NAALADase inhibition appears to affect glutamate only in pathological states', says Slusher. 'This is tremendously important. Basal glutamate does not seem to be affected, and we do not see the toxicities associated with other approaches to regulating glutamate. This potentially gives us a safe method to treat many disorders associated with excessive glutamate production.'

Future studies with NAALADase inhibitors

Stroke has presented various problems as a target for the first clinical application of NAALADase inhibitors, so the focus of research has now shifted to diabetic neuropathy and neuropathic pain. The high glucose levels found in patients with diabetes mellitus cause damage to the neurones and, although the mechanism is not currently understood, glutamate is known to be involved. 'We were really struck by the magnitude of protection offered in diabetic neuropathy models', Slusher said, 'both in the treatment of symptoms and in slowing disease progression.'

Guilford hopes to file an investigational new drug (IND) application for its lead NAALADase inhibitor compound for diabetic neuropathy and neuropathic pain by the end of 2000.

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Jo Whelan

Current approaches to multiple parallel synthesis

Approximately ten years ago, it was thought that organic chemistry was a mature science. Since then, however, chemistry has been rejuvenated with a technological revolution. This has been at great expense to the pharmaceutical and biotechnological industries and, so far, does not appear to have produced all the rewards it promised when combinatorial chemistry (CC) techniques were first conceptualized. An overview of current approaches to CC was given at the *Developing a successful combinatorial chemistry strategy to deliver more drug candidates* conference on 6–7 December 1999 in London.

Multiple parallel synthesis

The promise of automation

The traditional approach to synthesizing compounds and finding leads was 'linear', involving yes/no decisions at every step. If the wrong route was taken, one would return to the last stage and try a different route. Now, CC techniques enable parallel synthesis of compounds, which can generate typically 100-fold more compounds and, hence, 100-fold more data. Furthermore, it will enable the concurrent visualization of all the possible routes, enabling the correct decision to be made immediately. As highlighted by Geoff Lawton (Roche Products, Welwyn Garden City, UK), although this might actually slow down the process because the decision is more complicated, the final decision should be better.

Previously, projects were only started if there was a rationale for altering the chemistry of a particular ligand. Now, libraries can be screened and leads found rapidly. CC can increase the speed of lead generation and optimization and enable early low-cost target validation, as well as invalidation. However, to make automation beneficial, it is important to plan how to effectively use the 'saved' time, as it is very easy to waste it

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on non-value-added jobs. As CC can produce a wider range of exemplified compounds, a wider level of patent protection is also possible.

The main bottlenecks

In the classical techniques, the main bottlenecks were analysis [which has been overcome with the advent of LC–MS (liquid chromatography–mass spectrometry)] and purification (which is starting to be overcome using automated LC–MS purification). Now, with the synthesis of large numbers of compounds, the rate-limiting step is the data manipulation and assimilation. Many companies are also complaining of the difficulty in finding adequately trained IT staff to deal with data management problems. Another possible reason for the slow progress with CC is the necessity for continuous training of researchers to ensure that they are familiar enough with the rapidly changing computer technology to enable them to put it to full use.

Other potential drawbacks of automation, as highlighted by Andy Merritt (GlaxoWellcome, Stevenage, UK), included the need for expert support that is flexible and can turn around problems rapidly, as with fully automated systems, one breakdown will stop the whole process. Merritt also suggested that one key feature of any automation system is that the data is totally transferable and interchangeable between different systems, so that the data is only keyed in once to reduce time and mistakes. Because of the large variation in appropriate technologies for each company and for each project, automation systems are now being developed and built to the requirement of the customer, slotting together the appropriate components for the project without excessively increasing the cost.

Diversity

The diversity of a library is often one of the most important factors in determining its effectiveness. Diversity is measured by descriptors such as CLogP (a measure of lipophilicity), pKa, MW and, sometimes, molecular volume. Clusters can be used to examine the quality of a set of compounds in a library or a collection. Intra-cluster variance can then be used to determine the number of compounds necessary to fill a space within the cluster.

Ian Matthews (Chemovation Ltd, North Heath Lane, UK) suggested that a diverse library might be preferable earlier in the discovery process, while a more focussed library would be preferable later on for confirming a hit, and for changing a hit into a lead and a lead into a development compound. Furthermore, focussed libraries can add physicochemical data to hits, which can then be used to strengthen a patent application. One suggestion was that by starting with a diverse range of only a few compounds, the gaps around the selected interesting compounds could then be filled, reducing the total number of compounds synthesized.

Hugo Kubinyi (BASF, Ludvigshafen, Germany) suggested that larger libraries were more likely to produce hits, purely because of their size, and also enable easier derivation of SARs and/or QSARs, enabling a better patent coverage. However, a library 1000-fold larger than a smaller one would not produce 1000-fold as many hits because it would not be 1000-fold more diverse. By contrast, smaller libraries require much lower automation and much less effort for reaction optimization and production (e.g. liquid-phase extraction can be used instead of solid-phase extraction). In addition, several small libraries based on different templates generate much greater diversity than a single large library. Furthermore, with newer techniques to predict the properties of families of proteins becoming available, there is a general shift from using the larger libraries to screening smaller libraries of well-characterized compounds against one particular target and, if no hit is found, then screening the library against other targets.

Library design

Libraries can be designed based on chemical structure using either scaffolds (rigid structures with no pharmacological activity themselves on which sequences such as dimers and trimers can be 'hung') or morphates (amorphous backbones), or can be based on known pharmacophores using templates, or on targets using therapeutic or gene families. However, it was argued that the scaffold used was of crucial importance as some very large libraries can be relatively ineffective if based around a 'poor' scaffold. It is also important not to forget the quality and purity of the library when increasing the number of compounds in a library.

Another factor in the design of a library is whether the compounds should be drug-like. Using a library of drug-like compounds could increase the likelihood of any new lead also being drug-like. However, this lead is also more likely to be similar to a currently known compound class, reducing the chances of finding totally new classes. Lawton demonstrated this using the neuramidase inhibitors. These compounds are based on sialic acid, which contains a hydrophilic glycerol side-chain, which is present in zanamivir (Relenza, GlaxoWellcome). Hence, oseltamivir (Tamiflu, Roche, Basel, Switzerland) might have been missed, as it contains a hydrophobic side-chain by the rotation and the moving of the glutamic acid group by one position. Conversely, Hugo Kubinyi (BASF AG) highlighted that chemically similar compounds can have very different biological activities [such as promethazine (histamine H_1-receptor antagonist), chloropromazine (dopamine-receptor antagonist) and imipramine (uptake blocker)], and this biological 'similarity' depends greatly on the target[1].

Another issue is whether to test single

compounds or synthetic mixtures of compounds, the latter method having the advantage of requiring fewer tests, especially for pharmacokinetic evaluations, which are especially expensive. However, it appeared that the majority of companies are now generally using single compounds. Few companies still routinely using mixtures were Affymax (part of GlaxoWellcome) and Servier (Paris, France), where they use the technique for peptide libraries.

Multiple parallel solution synthesis techniques

Multiple parallel solution synthesis can be used for simplified reaction work-up, can be automated, can handle toxic, volatile or odorous compounds, can be used with high-throughput purification techniques, and excess resin-bound reagents/scavengers can be used to force the reaction to completion. This technique therefore has all the advantages of solution-phase chemistry, but can be run in parallel.

Resin-based reagents and scavengers

Mark Bradley (University of Southampton, UK) gave an overview of the use of resin-based reagents and scavengers. These can be divided into two groups. Gel resins are lightly cross-linked, have good reaction kinetics and a high-loading capacity, are not fragile and have a low MW cut-off. Here, the chemistry takes place by diffusion through the bead to the site of reaction and, hence, the smaller the bead, the faster the reaction. One major disadvantage of this method is that, as the bead volume is mainly solvent, extensive swelling and expansion occurs, sometimes to up to tenfold its own volume.

By contrast, macroreticular/macroporous resins have a well-defined surface area, are full of pores (where the chemistry occurs), are easy to filter because of their rigidity, have a large pore size, the resin volume remains fairly constant during solvent changes, and small solvent volumes are sufficient to wash the resin. The main disadvantages of this technique are relatively poor loadings, their brittleness, possible pore blockage, and relatively poor reaction yields as only the pore surface is accessible. Hence, it is important to balance accessibility into the bead with reaction on the bead.

Linker strategies for solid-phase chemistry

Shankar Balasubramanian (University of Cambridge, UK) gave an excellent presentation on the use of linker strategies for solid-phase chemistry. The ideal criteria for a linker is that it is chemically stable and compatible with the synthesis mechanism, selectively cleavable, easily separable, clean, fast, efficient and cheap, the first two aspects being the hardest to achieve. When linkers are used for screening, the release process must controlled, and the cleavage reagent must be absent, with aqueous/biocompatibility being preferable.

The most widely used linker is the Wang ester, which is usually used for releasing carboxylic acid compounds. Other value-added linkers include:

- Safety-catch linkers, which can be varied and the orthogonality of the linker tuned to the individual requirements of the reaction.
- Cyclative cleavage, which simultaneously finishes the chemistry and cleaves the resin. As long as the reaction was highly efficient in the earlier stages, the reaction will be very clean.
- A traceless linker, which replaces the linker bond with a hydrogen atom.
- Cleavage diversification adds diversity and functionality in the cleavage step and therefore reduces the number of necessary steps by at least one.
- Asymmetric induction uses a chiral linker, but should ideally be created so that the linker can be recycled, as this linker is very expensive.
- Partial release releases the material in a tiered fashion, and this new concept is very useful for single-bead screening of libraries.
- Biocompatible linkers are again becoming popular, especially with the increased use of screening using whole cells.
- Photolabile safety catches use chemical deprotection of the safety catch to render the compound sensitive to photocleavage. This might become a future method for releasing carboxylic acids as the photolysis reaction is relatively steady. If more than one safety catch is used, then they can be undone separately.

Summary

For CC to become successful, Lawton suggested that there must be a physiological and cultural shift in the way data is examined, from a stepwise method to looking at data in parallel. Meanwhile, Matthews highlighted the importance of retaining the experience, prejudices, imagination and creativity that has been present in the chemistry field, but which is in danger of being lost through the numerous mergers and acquisitions of the present day. This also relates to the understanding of the knowledge that CC creates, which might be lost through poor people-management by chemists and physicists, who do not necessarily make good managers.

Kubinyi summarized that, although CC is becoming increasingly important, it will not replace classical chemistry, as only a few syntheses and biologically active compounds can be covered by combinatorial libraries, and only a few techniques are suitable for, or require, automated syntheses. Furthermore, CC will not directly produce development candidates for clinical investigations, and hits identified by CC have not yet

been optimized and developed into marketed drugs because of the long time-scale of the drug development process. However, CC will remain a very important tool, which will increase knowledge, leading to an easier and faster search for new drugs.

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Rebecca N. Lawrence

β-Sheet breaking peptide might treat prion disease

Although human prion diseases are relatively rare, their effects on patients are devastating and there is currently no available treatment. A group of scientists from Serono Pharmaceutical Research Institute (Geneva, Switzerland), led by Claudio Soto, have now developed a novel peptide that might delay the progression of prion diseases by converting abnormal, destructive prion protein (PrP^{Sc}) back into normal prion protein (PrP^{c})[1].

Conversion of PrP^{c} to PrP^{Sc}

A key event in prion diseases is the conversion of PrP^{c}, a protein that contains many α-helices, into PrP^{Sc}, an isomer that contains a high proportion of β-pleated sheets[2]. PrP^{Sc} is an insoluble protein that is highly resistant to proteolytic digestion; these properties leading to its accumulation, particularly in the brain. Hence, the progression of prion diseases such as Creutzfeldt– Jakob disease (CJD), kuru, Gertsmann–Straussler–Scheiker disease and fatal familial insomnia in humans, and scrapie and bovine spongiform encephalopathy (BSE) in animals, occurs slowly at first but is then relentless.

Soto and colleagues at the New York University Medical Centre (NY, USA) reasoned that it should be possible to design molecules to disrupt the β-sheet-rich segments of PrP^{Sc} to inhibit their aggregation. Initially, they produced short synthetic peptides with sequence homology to PrP in the conserved region spanning residues 115–122, as this region appears to be important for the conversion of PrP^{c} to PrP^{Sc}. One of these peptides, iPrP13 (a 13-residue molecule with the sequence DAPAAPAGPAVPV), could induce the unfolding of the β-sheets and was termed a 'β-sheet breaker peptide'[1].

Effects of the iPrP13 peptide

In preliminary *in vitro* experiments, the protease susceptibility of partially purified PrP^{Sc} from scrapie-infected mouse brain was measured after incubation with different concentrations of iPrP13. Incubated samples showed significantly lower concentrations of the protease-resistant protein, indicating that a proportion of the PrP^{Sc} had been disrupted[1]. The extent of the change was dependent on the concentration of the peptide added; the concentration of protease-resistant prion protein fell from 100% to 49% at a onefold molar excess of iPrP13 but from 100% to 11.5% when a 1000-fold molar excess was used. Chemical analysis of PrP^{Sc} that had been incubated with the breaker peptide revealed a reduction in the β-sheet content of the molecule from 41% to 8% and an increase in the proportion of α-helix and random coil structure (Fig. 1).

When the higher concentration of iPrP13 was incubated with PrP^{Sc} derived from non-murine sources, including from one patient with CJD and one patient with new-variant CJD, a smaller reduction in the concentration of protease-resistant prion protein was seen, but it was still highly significant. 'Further development of β-sheet breaker peptides as therapeutic agents will require closely refining the peptide sequence', says Soto.

Soto then went on to test the iPrP13 peptide in a cellular model of familial prion disease[1]. Chinese hamster ovary cells overexpressing mutated PrP, that has the properties of PrP^{Sc}, were incubated with iPrP13 (100 $\mu g\ ml^{-1}$) for 48 h. When treated cells were exposed to proteinase K, the PrP^{Sc} signal was virtually undetectable, indicating a significant change in the level of PrP^{Sc} expressed. *In vivo* tests in mice infected with a sample containing partially purified PrP^{Sc} that had been pre-treated with an equimolar concentration of iPrP13

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