mosaic. In their review, Morphy *et al.* [1] propose 'ten aspirations' that they suggest could potentially be used to asses the feasibility of the design of a compound with potentially multiple targets. If these 'aspirations' are followed, with a particular emphasis on the recommended 'balanced modulation of several targets', the possibility that disease conditions could lead to targets having different (patho)physiological distributions, different densities, different cellular environments and different adaptations (e.g. internalization, receptor coupling and receptor upregulation) must be considered. Therefore, of the ten recommendations listed, which encompass topics that are generally applicable to many of the requirements in drug development, particular importance must be placed on the need for a clear understanding of the *in vitro–in vivo* relationship of a compound; an evident relationship between *in vitro* activities, *in vivo* activities and clinical profile is what everybody in drug development desires but hardly ever achieves. Successful drug development depends on interdisciplinary work between specialists in the medicinal chemistry, molecular modelling, crystallography, biochemistry, pharmacy, pharmacology and clinics fields, as well as with those working in life sciences. In general, this seems to be the case in current drug development, but there is an even greater need for cooperation when multiple targets per molecule is the focus of research.

A change in direction for the route to successful drugs from a single target (monogamy) to selected multiple targets (strategic promiscuity) appears to be a highly promising and potentially beneficial therapeutic strategy. However, a superior understanding of the causes, and their complexity, of the disease is essential.

Reference

1 Morphy, R. *et al.* (2004) From magic bullets to designed multiple ligands. *Drug Discov. Today* 9, 641–651

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Medicinal chemistry: new technologies and developments

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The Medicinal Chemistry Division of the American Chemical Society (http://www.chemistry.org) offered a rich menu of drug research papers at the 227th *American Chemical Society National Meeting* in Anaheim, CA, USA (28 March–1 April 2004).

The Chemetics™ technology developed by the Danish firm Nuevolution (http://www.nuevolution.com) appears to take the concepts of combinatorial synthesis and HTS one step further by using a hybrid of wet chemistry and molecular biology. Alex Gouliaev of Nuevolution described this technology as a wet chemistry process in which DNA-directed synthesis is performed in a single reactor to produce ultra-large small-molecule libraries that contain 10^8–10^{14} compounds. The starting materials are synthesized using standard organic synthetic chemistry before library formation, and could include most of the chemical structures that are currently used in medicinal chemistry.

New chemical entities could be isolated from the mixture of compounds by a selection process that mimics natural evolution. Gouliaev suggested that Chemetics™ could revolutionize drug discovery by enabling the one-pot synthesis and the screening of billions of drug-like small molecules in just a matter of weeks. Chemetics™ technology uses DNA-template sequences to direct the synthesis of small organic molecules. On incubation of the DNA-templates with oligonucleotides to which different drug fragments have been attached, the oligonucleotides bind to their complementary base in the DNA-template, which fixes the position of the drug fragment and facilitates their reaction. As a result of the close proximity effect, only drug fragments that are associated with the same complex will react. Thus, the end-result is that a specific DNA-template directs the synthesis of a particular molecule. Moreover, the structural diversity of this large library is much greater than that produced by current combinatorial

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chemistry methods. Next, the large library of small-molecule–DNA complexes generated can be passed through an affinity-chromatography column onto which a target of interest has been immobilized. Ligands bind to the target; small molecules that do not bind are washed off the column. The small-molecule ligands are then eluted and can be amplified by sequential amplification of the DNA by PCR and then Chemetics™ encoding. Alternatively, their structure could be revealed from the sequencing of the specific DNA-template that directs their synthesis.

The DNA-templates have three key functions – they direct small molecule synthesis, enable the elucidation of the structure of the DNA-attached small molecule and provide a means of amplification of the small molecule, without the need for the initial characterization of the DNA template sequence or the determination of the chemical structure of the small molecule.

The leads that are identified by column chromatography are then analyzed in standard downstream assays, including *in vitro* efficacy and *in vivo* efficacy assays, and absorption, distribution, metabolism, excretion and toxicity (ADMET) studies. Simpler assays are initially carried out to reduce the number of more expensive, more time-consuming assays that must be performed. The high degree of structural diversity of the initial drug lead candidates greatly increases the probability that some of these leads will pass all the assays. This high degree of chemical structural diversity in the compound library reduces the need for the repetition of the synthesis and screening steps and the SAR determinations that are a frequent feature of current combinatorial chemistry; the ultimate clinical candidate is already present in the initial compound library.

Because fewer syntheses, assays and SAR analyses need to be performed for the identification of clinical trial candidates, drug candidates are recognized more quickly. If the Chemetics™ technology delivers on this potential, it could significantly accelerate drug discovery and commercialization while simultaneously reducing drug development costs.

Antisense therapeutics

Charles Allerson of Isis Pharmaceuticals (http://www.isispharm.com) described Isis' development of antisense therapeutics. RNA interference (RNAi) provides a powerful mechanism for the inhibition of undesirable gene expression in mammalian cells. This inhibition prevents or reduces the production of disease-related proteins that are involved in disease.
A mechanism in the cell identifies an antisense drug that is hybridized to mRNA and dispatches a natural cellular enzyme to destroy the mRNA.
This destruction prevents production of the disease-causing protein. Antisense drugs are designed to interact specifically with the intended mRNA target with a high degree of specificity that can make antisense drugs both more effective and less toxic than traditional drugs.

The recent discovery that small-interfering RNAs (siRNAs) interact with protein components to form the RNA-induced silencing complex (RISC) that activates the release of the cellular enzyme has resulted in the widespread use of siRNAs in *in vitro* functional genomics (gene regulation). Use of siRNA to knock-down the message that is specifically carried by a gene, thereby reducing the protein level of the targeted genes, is increasingly employed in genetic knock-out technologies, dominant negatives and chemical inhibitors of protein activities. Hence, interest in siRNA technology continues to grow.

Methods for preparing siRNAs include chemical synthesis, *in vitro* transcription, siRNA expression vectors and PCR expression cassettes. Use of siRNAs for gene silencing is a rapidly evolving tool in molecular biology and this has triggered interest in their potential therapeutic use.

Various double-stranded siRNAs have significant potential in antisense therapeutics because RNAi that is triggered by siRNAs selectively reduces levels of targeted mRNA. However, the metabolic instability of currently known siRNAs renders them unsuitable for therapeutic use. Allerson reported that, although unmodified siRNAs are sufficiently robust for *in vitro* use, siRNA chemical structure modifications are required that concomitantly improve their pharmacokinetic properties and stabilize them against metabolic degradation to a level that is sufficient for *in vivo* use. Therefore, there is a need for a systematic understanding of the SAR of chemical modifications throughout siRNA that would have limited effects on RISC loading of the siRNA, but that would afford stability and retain biological activity.

Researchers at Isis reported the results of studies that were designed to fill this need. Charles Allerson's research team replaced the 2′-hydroxyl groups in siRNA duplexes either partially or completely with combinations of 2′-deoxy, 2′-*O*-methyl, 2′-*O*-(2-methoxyethyl) and 2′-fluoro chemical groups. These modifications produced siRNAs with improved target reduction activity and serum stability. Thazda Prakash (Isis) reported the results of the systematic scanning some of these chemical modifications, such as 2′-*O*-methyl, 2′-deoxy-2′-fluoro and 2′-*O*-methoxyethyl, which identified some structures that showed strong positional effects throughout the antisense strand.

Prasad Dande (Isis) reported results from the synthesis of 4′-thio-siRNAs and

the investigation of their ability to induce RNAi. The four 4′-thio-nucleosides were synthesized following published procedures, but some modifications were incorporated at key steps in the synthesis. After converting the 4′-thio-nucleosides into the corresponding phosphoramidites, they were incorporated into 20mer oligonucleotides. These modified oligonucleotides were converted into 4′-thio-siRNAs and then tested in an *in vitro* assay against a phosphatase and tensin homolog (PTEN) target. Reduced PTEN mRNA levels indicated that a number of these chemically modified siRNAs showed potent RNAi activity. Furthermore, the researchers also noted a strong correlation between the number and placement of 4′-thio residues and siRNA activity.

Obesity, diabetes and other inflammatory diseases

Obesity is a major factor in the onset of Type II diabetes, particularly in children and young adults. The melanocortin-4 (MC4) receptor is a promising target in the treatment of obesity and associated insulin resistance. Activation of the MC4 receptor has been shown to simultaneously suppress appetite and increase metabolic rate; activation of this receptor has resulted in significant weight loss in several animal models. Kilian Conde-Frieboes of Novo-Nordisk (http://www.novonordisk.com) reported the identification of novel types of MC4 receptor agonists. The strategy used was to mimic the key residue side chain functionalities of the potent cyclic heptapeptide agonist melanotan-II (MT-II) by suitable substitution on non-peptide scaffolds. The key residues of MT-II were identified to be D-Phe7, Arg8 and Trp9. Substitution of Ala for these amino acids (or D-Ala for D-Phe7) resulted in a >100-fold reduction in the binding affinity. Using this procedure, several new compound classes of MC4 agonist were discovered. The chemical structure was optimized by combinatorial synthesis using a focused library design strategy. Potent MC4 agonists were synthesized with an *in vitro* activity (EC_{50}) of 20 nM in a cAMP assay. In our assay, MT-II and α-melanocyte stimulating hormone have activities (EC_{50}) of ~1 nM and ~100 nM, respectively. (EC_{50} is the drug concentration that provokes a response that is halfway between the baseline response and the maximum response).

Although the macrophage migration inhibitory factor (MIF) is known to play an important role in several inflammatory and autoimmune diseases, little is known about the role of this proinflammatory cytokine in Type 1 diabetes. MIF mRNA expression is upregulated in non-obese diabetic mice [1]. Yousef Al-Abed (North Shore-Long Island Jewish Research Institute; http://www.northshorelij.com) and his colleagues at the Institute for Biological Research (Siniša Stanković; http://www.ibiss.bg.ac.yu) and the University of Catania (http://www.unict.it) reported that MIF protein concentration was significantly elevated in islet cells during the development of experimental diabetes (induced in mice by multiple low doses of streptozotocin). A significant reduction in the histopathological changes in pancreatic islets suppressed the development of hyperglycemia. This resulted from reducing MIF activity with neutralizing antibodies against MIF or the pharmacological MIF inhibitor (*S*,*R*)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester (ISO-1). Al-Abed attributed these beneficial effects to the reduced proliferation and adhesion of autoreactive lymphocytes and the downregulation of inducible nitric oxide synthase (iNOS) expression. Other possible causes were nitrous oxide and tumor necrosis factor-α (TNF-α) secretion by islet cells and peritoneal macrophages. Thus, MIF has a crucial role in the pathogenesis of Type 1 diabetes and inhibition of this function could provide a new strategy for attenuating the autoimmune process.

Matthew Laufensweiler of Procter & Gamble Pharmaceuticals (P&GP; http://www.pgpharma.com) reported that researchers at P&GP are developing 4-aryl-5-pyrimidinyl-based cytokine inhibitors to reduce inflammation that is associated with diseases such as osteoarthritis, rheumatoid arthritis and Crohn's disease; proinflammatory cytokines, and in particular TNF-α and interleukin 1-β, have been implicated in the exacerbation of these conditions. Laufensweiler reported that novel spiroketal [5,5]-bicyclic pyrazolones and substituted [5,5]-bicyclic pyrazolones inhibited TNF-α production. Some of these compounds exhibit low nanomolar activity against lipopolysaccharide induced TNF-α production in T-helper precursor-1 cells. Michael Clark of P&GP reported that TNF-α inhibitor studies demonstrated that several of these compounds exhibit good oral bioavailability in rats.

Summary

The research presented at the conference is in progress, but could result in significant advances in drug therapy. However, it will still be years before any drug candidates that are synthesized using Chemetics™ technology enter clinical trials. Similarly, the continued development of SARs for modified siRNAs is needed before optimized compounds can be identified and considered for clinical trials. Furthermore, extensive research is required before clinical trials of specific diabetes treatments that are based on MIF strategies can be performed. By contrast, research suggests that clinical trials of cytokine inhibitors for the treatment of arthritis are imminent.

Reference

1 Waeber, G *et al.* (1997) Insulin secretion is regulated by the glucose-dependent production of islet β cell macrophage migration inhibitory factor. *Proc. Natl. Acad. Sci. U. S. A.* 9, 4782–4787