News in brief

Targets and mechanisms

Structure of promiscuous molecule determined

The structure of a key molecule in the liver that regulates the metabolism of >60% of drugs has been determined by chemists at the University of North Carolina (Chapel Hill, NC, USA) and GlaxoSmithKline[1]. The molecule, pregnane X receptor (PXR) is a nuclear receptor and transcription factor that regulates cytochrome P450-3A (CYP3A), controlling the expression of proteins that metabolize many of the drugs we take.

Potentially dangerous drug–drug interactions can occur when PXR is activated by xenobiotics and have caused certain powerful AIDS drugs (including idinavir) and transplant drugs (including cyclosporin) to become less effective. The interactions have also led to 'St John's wort babies', where the unregulated herbal antidepressant caused PXR to make oral contraceptives ineffective. Furthermore, a constituent of the herb, hyperforin, binds to PXR and stimulates production of CYP3A4, which metabolizes compounds in the liver including contraceptives.

Most interactions in the body are highly specific but PXR is promiscuous, binding to several drugs and toxins of varying sizes including rifampicin, taxol and the abortion pill (RU486). The team determined the structure of the ligand-binding domain of the human PXR alone and in combination with the cholesterol-lowering drug SR12813 and discovered that it contains a large, smooth pocket that can accommodate a variety of compounds. Several polar residues within the mostly hydrophobic cavity were shown to specify a directed promiscuity and to mediate the binding of SR12813 in at least three different ways.

Interestingly, although PXR is chemically promiscuous, other groups have shown that murine PXR binds to a different set of chemicals than human PXR, suggesting that PXR has evolved to face the different challenges encountered by humans and mice. Only a few chemical groups in PXR are responsible for this selectivity: if just four amino acids are changed in murine PXR, to the corresponding residues in human PXR, the receptor can be 'humanized' in its response to specific chemicals.

1 Watkins, R. *et al*. (2001) The human nuclear xenobiotic receptor PXR: structural determinants of directed promiscuity. *Science* 10.1126/science.1060762 (http://www.scienceexpress.org)

New amino acid could treat schizophrenia and Parkinson's disease

An amino acid, neo-tryptophan, which could provide a novel way of treating schizophrenia and Parkinson's disease, has just been patented in the US by the Mayo Foundation for Medical Education and Research (Rochester, MN, USA). The patent covers the amino acid and any peptide that contains it.

It has been suspected since the 1980s that neurotensin (a neuropeptide comprised of 13 amino acids) acts as an endogeneous anti-psychotic agent. This led to the idea that neurotensin analogues could be developed as novel anti-psychotic drugs that would activate neurotensin receptors but, unlike neurotensin itself, would be degraded much more slowly increasing their duration of action. The Mayo researchers, led by Elliott Richelson, systematically changed the sequence of the 8–13 amino acid portion of the molecule.

In the process of developing ~80 different neurotensin 8–13 analogs, they elucidated the structure of a substitute for the tyrosine molecule in the 11th position of the molecule that fits better into the human receptor than the rat receptor. They created an amino acid that had the correct 3D configuration to replace the tyrosine and was similar to tryptophan, and was named neo-tryptophan. When substituted into the peptide, the binding of the peptide to the human receptor was markedly increased.

The group are seeking Food and Drug Administration (FDA) approval to begin testing a neo-tryptophan-containing peptide as a drug for treating schizophrenia and Parkinson's disease. The drug also has analgesic properties and lowers body temperature so could potentially be used to preserve neurons in the brain after an anoxic event such as cardiac arrest. It is hoped that clinical trials will be underway by late 2001.

Success in sight for gene therapy drug candidate

Researchers have successfully reduced the growth of blood vessels in models of macular degeneration and diabetic retinopathy using gene therapy. Macular degeneration and diabetic retinopathy are the two leading causes of blindness in developed countries and are characterized by excessive new blood vessel formation (angiogenesis), which results in exudation, bleeding and scar formation in the retina and the choroid and, ultimately, visual impairment and blindness.

Researchers from the Wilmer Eye Institute (Baltimore, MD, USA) and Genvec (Gaithersburg, MD, USA) have administered a gene that encodes pigment epithelium-derived factor (PEDF) to the eyes of animals with macular degeneration of diabetic retinopathy. They found that angiogenesis was significantly reduced in animals treated with the gene.

PEDF is the most potent endogenous inhibitor of blood vessel growth in the eye, and these latest findings have shown that it is an effective inhibitor of both retinal and choroidal neovascularization. Further, this study has also demonstrated that gene therapy is a valid method for achieving sustained intraocular levels of PEDF. Genvec is now developing PEDF as a gene therapy to treat macular degeneration and diabetic retinopathy, using an adenovector vehicle to deliver and produce the growth factor. Gerald Chader, CSO of The Foundation Fighting Blindness (Owings Mills, MD, USA), believes that: 'If this approach is demonstrated to be effective in subsequent human clinical trials, it could revolutionize the treatment of these two diseases.'

New protein might provide cure for Alzheimer's disease

A protein, termed humanin, has been discovered that can stop brain cell death associated with Alzheimer's disease. Different types of familial Alzheimer's disease genes and Aβ amyloid cause death of neuronal cells. However, the research group led by Ikuo Nishimoto (Keio

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Clinical trials

Placebo use in hypertension trials ethical

Using placebo drugs in studies of hypertension is ethical and safe in the short term, concludes a recent article in *Science*[8].

The review of 25 hypertension studies conducted in the late-1990s at Duke University Medical Center (Durham, NC, USA) found that placebo-treated patients do not suffer greater adverse health-effects than patients receiving active treatments. Adverse events among the 6409 participants included stroke, myocardial infarction, congestive heart failure and death.

'The general practice of using placebo drugs to test the effectiveness of new drugs is an acceptable and useful research tool under the right conditions,' said Robert Califf, Director of the Duke Clinical Research Institute. 'It provides a more accurate test than drug-to-drug analyses,' says Califf, 'because no active treatment is used at all. As a result, sample sizes can be smaller to obtain the same statistical significance.'

'Clinical investigators, however, must be able to show that withholding active therapy from patients for a short time is unlikely to result in physical harm,' said Sana Al-Khatib, lead author of the study. 'Informed, voluntary patient consent must also be obtained and patient progress closely monitored,' he continued.

Opponents of placebo research claim that it is dangerous and unethical to withhold effective treatments for diseases where therapies exist. In the case of hypertension, this could lead to unnecessary cases of stroke, coronary heart disease or premature death, the article claims.

8 Al-Khatib, S.M. *et al.* (2001) MEDICINE: Placebo-controls in short-term clinical trials of hypertension. *Science* 292, 2013–2015

First human dose Phase I trial completed for oral heparin

The results of the first human dose Phase I trial for oral heparin using SNAD as a delivery agent have been promising. The trial, completed by Emisphere Technologies (Tarrytown, NY, USA), was a safety and tolerability study of the oral formulation of heparin developed using the company's proprietary delivery agent, sodium *N*-[10-(2-hydroxybenzoyl)amino]decanoate (SNAD).

SNAD is structurally related to another delivery agent that was developed by Emisphere, sodium *N*-[8-(2-hydroxybenzoyl)amino]caprylate (SNAC), which is currently in Phase III testing as an oral delivery agent for heparin. SNAD was developed after modification to the SNAC family of delivery agents resulted in favourable structure–activity relationships.

In this human Phase I study, two groups of 12 volunteers were given increasing doses of SNAD either alone, or in combination with unfractionated heparin, as an oral dose. A dose-dependent increase in anti-coagulation markers, aPTT and anti-factor Xa, was observed and the formulation was well-tolerated, with no serious adverse effects being reported. Emisphere intend to use SNAD as an agent for delivering both fractionated and low-MW heparin, and are hoping to extend the use of heparin from 1–2 weeks to >30 days in a home setting for the preventative treatment of deep vein thrombosis (DVT).

University, Tokyo, Japan)[2], discovered that humanin (which is naturally produced at the back of the brain) encodes a short polypeptide that abolishes such cell death. This peptide is a molecular clue for the development of new therapeutics for Alzheimer's disease and animal testing of the compound should begin soon.

One of the features of Alzheimer's disease is that patients gradually lose memory and attention functions. Recently, Titia Sixma and coworkers (Dutch Cancer Center)[3] reported a three-dimensional model of a brain protein produced by glia cells in the CNS that helps to transmit messages between brain cells that control these functions. Also, August Smith and coworkers (Vrije University) have reported that the protein acts as part of a receptor for acetylcholine, a signalling chemical involved in memory. By knowing the structure of the binding site, chemists can design drugs that bind to the appropriate receptors. Whether humanin has the necessary three-dimensional chemical structure to bind with these receptors remains to be determined.

2 Hashimoto, Y. *et al*. (2001) A rescue factor abolishing neuronal cell death by a wide spectrum of familial Alzheimer's disease genes and Aβ. *Proc. Natl. Acad. Sci. U. S. A.* 98, 6336–6341

3 Brejc, K. *et al*. (2001) Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors. *Nature* 411, 269–276

Quorum sensing – a structural genomics approach

The crystal structures of three enzymes that are involved in the virulence of many pathogenic bacteria, have been elucidated at Structural Genomix (SGX, San Diego, CA, USA)[4]. These enzymes belong to the LuxS family of proteins, which is involved in the quorum-sensing pathway, an environmentally stimulated communication system in bacteria. Responses to external stimuli could be contributing factors to the increasing resistance of these pathogens to antibiotics, hence the importance of elucidating their molecular mechanisms and structure–activity relationships in the development of novel antimicrobials.

The group at SGX determined the crystal structures of LuxS proteins from three bacterial species, with resolutions between 1.8 and 2.4 Å, using an X-ray crystallographic structural-genomics approach. Analysis of the three enzymes in parallel could lead to the full determination of their precise enzymatic function.

The enzymes take the form of a novel α–β barrel fold, a homodimer is seen and the structure of the binding site has been solved. A metal ion identified as zinc was observed binding to a Cys–His–His triad, and methionine binds to the protein close to the zinc ion at the dimer interface. The hypothesis for the *in vivo* activity of these enzymes is that they bind to a methionine analog (*S*-ribosylhomocysteine) and the zinc atom cleaves the ribose ring to, ultimately, form the quorum-signalling molecule, autoinducer-2.

4 Lewis, H.A. *et al.* (2001) A structural genomics approach to the study of quorum sensing: crystal structures of three LuxS orthologs. *Structure* 9, 527–537

Inactivation of K^+ channels revealed by structural studies

The discovery of how K^+ channels can close rapidly to regulate the firing of neurons was made recently by a research team at Yale University (CT, USA)[5]. K^+ channels are membrane proteins involved in maintaining the concentration of K^+ ions across the cell membrane, which is crucial for the maintenance of heart beat, insulin release and nerve signal generation.

The team, led by the Howard Hughes Medical Institute (HHMI) investigator, Roderick MacKinnon (Rockefeller University, NY, USA), used X-ray crystallography of K^+-channel mutants to show that one of the four 'tails', or inactivation gates, at the N-terminus of the channel can slide into the pore, thus inactivating the channel. The central cavity and inner pore of the channel form the receptor site for both the inactivation gate and small-molecule inhibitors, indicating that the cavity could be the site-of-action of drugs that alter the function of the K^+ channel.

Fellow HHMI investigator Richard W. Aldrich (Stanford University, CA, USA) says, 'These findings unify the functional work on inactivation over the past 50 years, and settle a controversy about how the N-terminal peptide could inactivate the channel.'

5 Zhou, M. *et al.* (2001) Potassium channel receptor site for the inactivation gate and quaternary amine inhibitors. *Nature* 411, 657–661

Can vitamin C fight the big C?

Vitamin C induces the decomposition of lipid hydroperoxides to endogenous genotoxins, as discovered by a research group at the Center for Cancer Pharmacology at the University of Pennsylvania (PA, USA; Ref. 6). Usually, this breakdown is catalyzed by transition-metal ions but this study has shown vitamin C to be twice as effective as the metal ions at inducing genotoxin formation. The genotoxins take the form of DNA-reactive bifunctional electrophiles, one of which causes a highly mutagenic lesion found in human DNA.

However, these mutations can be repaired and this does not imply that vitamin C causes cancer. Traditionally, this vitamin, which is a DNA-protecting antioxidant, is thought to be implicated in the prevention of cancer formation. Indeed, the wisdom of eating a balanced diet rather than a single antioxidant, is expounded. Ian Blair, who led the research, says, 'It's possible that vitamin C isn't working in cancer prevention studies because it's causing as much damage as it's preventing, but that's really speculation at this point.' These findings could help to explain the lack of efficacy that vitamin C has shown thus far as a cancer chemopreventative agent.

6 Lee, S.H. *et al.* (2001) Vitamin C-induced decomposition of lipid hydroperoxides to endogenous genotoxins. *Science* 15, 2083–2086

Miscellanous

Appeal denied as Eli Lilly loses Prozac patent

The US Court of Appeals Panel has reaffirmed its earlier ruling invalidating Eli Lilly's 2003 patent on the anti-depressant drug Prozac, it was announced by Barr Laboratories (Pomona, NY, USA) recently.

Eli Lilly (Indianapolis, IN, USA) requested that the Barr appeal on the company's 'double-patenting claim' (referring to the December 2003 patent protecting Prozac) be reheard. However, the original panel of judges reaffirmed, on a different legal basis, its previous conclusion that the patent is invalid.

'The Court of Appeals decision…will result in hundreds of millions of dollars in savings to consumers,' said Bruce Downey of Barr. Barr expects to launch fluoxetine (the generic name for Prozac) as a 20 mg capsule product on 2 August 2001, when Eli Lilly's paediatric exclusivity expires. The company expects to receive 180 days of generic exclusivity for its 20 mg capsule as a result of being the first to file under the Hatch-Waxman Act.

However, Pharmaceutical Resources (Spring Valley, NY, USA) announced recently that it also anticipates receiving all, or nearly all, of the six months exclusivity granted by the FDA for its various fluoxetine products. The company intends to market various forms of the drug later this year.

New center for immune research

A private donation of US$7.7 million to the Hospital for Special Surgery (HSS; New York, NY, USA) will give New York a new centre that will focus on research into the immune-system disease Lupus, it was announced recently.

The new Mary Kirkland Center for Lupus research aims to identify the genetic and immune-system determinants of the disease, systemic lupus erythematosus, a systemic autoimmune disease that can effect the skin, joints, kidneys and brain. The centre will conduct patient-focused research to understand the most important features of the disease, and to improve the quality of life for sufferers, 90% of which are women.

Fellowship scheme to support oncology research

A fellowship scheme to support 'translational' oncology research was announced recently at the first *European Organization for Research and Treatment of Cancer* (EORTC) *Translational Research Meeting* in Belgium.

'Translational' oncology aims to link research with clinical practice by, for example, preclinical studies and early clinical trials that validate novel therapeutic or diagnostic agents, or laboratory studies that aim to improve the use of established clinical agents, such as growth factor agonists.

The scheme is being funded by an educational grant from AstraZeneca. Medical practitioners or scientists who intend to specialize in oncology research are eligible to apply for a two-year fellowship, which will be undertaken in EORTC Affiliated Institutions in association with ongoing or planned EORTC studies. Further details of how to apply can be found at the EORTC website (http://www.eortc.be).

Evidence of how to mend a broken heart

Heart muscle cells have been shown to regenerate after myocardial infarction[7]. Scientists at New York Medical College

(Valhalla, NY, USA) investigated the levels of mitosis in myocytes after myocardial infarction in humans.

The level of cell division was measured by labelling a mitosis-associated antigen, Ki-67, and this measurement was then used to calculate the mitotic index (ratio of dividing cells to non-dividing cells). Morphological signs of cell division, such as mitotic spindles, contractile rings, karyokinesis and cytokinesis, were also recorded.

In infarcted hearts, an increased level of mitosis was recorded in cells adjacent to the infarct, and morphological evidence of mitosis-associated events was observed.

This challenges the previous conception that the heart is a post-mitotic organ. Claude Lenfant (Director of the National Heart, Lung and Blood Institute, NHBLI; Bethesda, MD, USA) comments, 'With this landmark study, we have a new understanding of the heart that opens up the possibility of repairing heart muscle damage after a heart attack.' David Finkelstein, director of basic cardiovascular research at the National Institute on Aging (NIA; Bethesda, MD, USA) adds: 'This finding, if confirmed, may begin to clarify how hearts respond to the normal insults of aging through previously undetected repair mechanisms.'

7 Beltrami, A.P. *et al*. (2001) Evidence that human cardiac myocytes divide after myocardial infarction. *New Engl. J. Med.* 344, 1750–1757

Mergers and acquisitions

BMS to acquire DuPont Pharmaceuticals

Bristol-Myers Squibb (BMS; New York, NY, USA) is to acquire DuPont Pharmaceuticals Co (Wilmington, DE, USA), a wholly owned subsidiary of DuPont, for $7.8 billion. This acquisition is hoped to help BMS in their growth strategy, which is to double sales, earnings and earnings-per-share between the end of 2000 and 2005.

Peter Dolan, President and CEO of BMS, said: 'Several of the promising compounds in [DuPont's] R&D pipeline have novel mechanisms of action and blockbuster potential. They have potential to address serious unmet medical needs, and contribute to the acceleration of our pharmaceutical sales and earnings growth in the future.'

The agreement is subject to approval by the appropriate regulatory agencies and customary closing conditions and should be completed by the end of 2001. If this transaction goes ahead on time, it is expected to be accretive to earnings per share (EPS) beginning in 2003. BMS anticipates recording a one-time, in-process R&D write-off and restructuring liability of US$2–3 billion.

Richard Lane, President of Worldwide Medicines Group and Executive Vice-President of BMS, will oversee the integration of DuPont Pharmaceuticals and BMS. The integration team will be managed by Rick Whinningham, President of Immunology and Oncology and Global Marketing at BMS Worldwide Medicines Group.

Sequenom and Gemini Genomics merge

Sequenom (San Diego, CA, USA) and Gemini Genomics (Cambridge, UK) have announced that they will merge their businesses in a stock-for-stock exchange. The merger is intended to significantly increase the ability of Sequenom to perform disease gene association and genetic marker validation studies, thereby providing a pipeline of validated genes for downstream development of diagnostic and therapeutic products.

The agreement is expected to close in the third-quarter of 2001 at which point Sequenom hopes to issue 12.9 million shares and assume all outstanding options and warrants. The transaction is still subject to approval by the shareholders of both companies as well as by the High Court of Justice in England and Wales. The two companies were already in an existing collaboration, which led to the identification of two novel cardiovascular disease genes using Sequenom's technology platform and Gemini Genomic's collection of diverse populations and clinical data.

Toni Schuh, CEO of Sequenom said: 'It is clear that the combined technological capabilities, clinical data and scientific expertise will give the company the ability to offer an enhanced suite of products and resources.' Helmut Schuhsler, Chairman of Sequenom, will remain Chairman of the new company; Michael Fitzgerald, Chairman of Gemini Genomics, and another nominee from Gemini will join the board of Sequenom; and Paul Kelly, CEO of Gemini, will become Executive Vice-President and lead the Biotherapeutics Division of the new company.

Biofocus acquires Cambridge Drug Discovery

Biofocus (Sittingbourne, Kent, UK) has announced it will acquire Cambridge Drug Discovery Holdings Ltd (Cambridge, UK) for £27.5 million. The acquisition is hoped to broaden the platform of early-stage drug discovery services of Biofocus to biotechnology and pharmaceutical clients by combining chemistry with biology.

Under the terms of the agreement, Biofocus will offer ~17.5 shares for each Cambridge Drug Discovery share. Ian Kent, Chairman of Cambridge Drug Discovery, will join Biofocus as a non-Executive Director and Jonathan Treherne, CEO of Cambridge Drug Discovery, will become Commercial Director at Biofocus. To bring the board structure in line with industry best practice, Alan Clabon, Paul Doyle, John Harris and Valerie Rose will resign from the Board of Biofocus and join a new management board, which will have responsibility for the operational management of the enlarged group.

Celera to merge with Axys

Celera Genomics (Rockville, MD, USA) has announced a definitive merger agreement with Axys Pharmaceuticals (South San Francisco, CA, USA). Celera will acquire Axys, a company specializing in small-molecule therapeutics, in a stock-for-stock transaction to expand the drug discovery side of their business.

'[Axys] has impressive capabilities in medicinal chemistry, high-throughput screening, and pharmacology that should enhance Celera's therapeutic discovery platforms,' said Craig Venter of Celera Genomics. Relationships between Axys and pharmaceutical manufacturers such as Merck and Co. (Whitehouse Station, NJ, USA), Aventis Pharmaceuticals (Parsippany, NJ, USA) and Bayer AG (Leverkusen, Germany) also made the company an attractive target.

Axys shareholders can expect to receive approximately US$4.65 per share, payable in Celera common stock. The transaction is subject to approval by Axys shareholders and regulatory approval.

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