against ovarian cancer in clinical trials. The heteroarotinoids inhibited the growth of all cancer cell lines (low micromolar IC_{50} values), with weak activity against normal and benign cells, when comparing an ovarian cancer cell line (OVCAR-3), a borderline ovarian tumour (O1), a benign ovarian cyst (O3) and normal endometrial cells. Growth inhibition was associated with cell loss (apoptosis) and generation of reactive oxygen species, and was found to be independent of retinoic acid receptor activation. Compound **iiia** (X=S, Z=NO2, R,R'=H) is currently in preclinical development for cancer prevention and treatment (National Cancer Institute; http://www.nci.nih.gov).

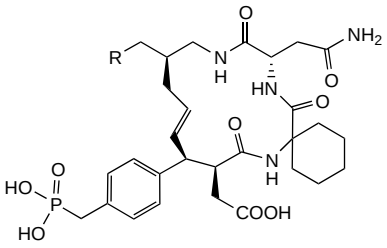
1 Hanahan, D. and Weinberg, R.A. (2000) The hallmarks of cancer. *Cell* 100, 57–70
2 Capdeville, R. *et al.* (2002) Glivec (STI571, imatinib), a rationally developed targeted anticancer drug. *Nat. Rev. Drug Discov.* 1, 493–502
3 Wu, S. *et al.* (2004) Induction of apoptosis and down-regulation of Bcl-XL in cancer cells by a novel small molecule, 2-[[3-(2,3-dichlorophenoxy)propyl]amino]ethanol. *Cancer Res.* 64, 1110–1113
4 Liu, S. *et al.* (2004) Synthesis of flexible sulfur-containing heteroarotinoids that induce apoptosis and reactive oxygen species with discrimination between malignant and benign cells. *J. Med. Chem.* 47, 999–1007

Andrew D. Westwell
Andrew.Westwell@nottingham.ac.uk

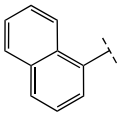
Ultrapotent Grb-2 SH2 domain-binding ligands

It is known that phosphorylation at particular phosphotyrosine (pTyr) residues serves as an on–off switch for the Src homology 2 (SH2) domain of proteins. This non-catalytic domain recognizes short peptide motifs bearing pTyr; the binding to pTyr sites can thus affect SH2-containing proteins in multiple ways. The growth factor receptor bound protein 2 (Grb-2) is an SH2 domain-containing docking module that represents an attractive target for anticancer therapeutic intervention.

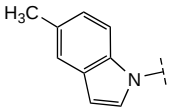
On the basis of the preferential binding of Grb-2 SH2 domain to pTyr-X-N containing sequences in a bend conformation, a series of macrocyclic peptide mimetics carrying a hydrolitically stable phosphonomethylphenylalanine instead of the pTyr residue was prepared. Thus, derivative **va** was found to have high



(va) R =



(vb) R =



Grb-2 SH2 domain binding potency in both extracellular (IC_{50} = 2 nM) and whole cell assays (IC_{50} ≥1 μM) [5] despite the presence of anionic functionalities, which often represent a limitation for cell membrane transit. In particular, the presence of the acidic functionality at the pTyr mimetic α position is essential to obtain high potency in whole-cell assays.

Then, on the basis of molecular modeling studies, **vb** was synthesized, with the aim to enhance the hydrophobic interactions with the SH2 domain. The resultant compound showed a *K*d value of 92.7 pM; thus, **vb** is significantly more potent that **va** (**va**: *K*d = 0.91 nM) and is the highest affinity agent yet reported for a synthetic Grb-2 SH2 domain-binding ligand [6]. It is also significantly more active than compound **va** in cellular assays and is able to elicit antimitogenic effects in growth-factor-driven breast cancer cells at noncytotoxic submicromolar concentrations [6]. These results indicate a high potentiality of this class of signal-transduction altering compounds as therapeutic agents.

5 Wei, C-Q. *et al.* (2003) Macrocyclization in the design of Grb-2 SH2 domain-binding ligands exhibiting high potency in whole cell systems. *J. Med. Chem.* 46, 244–254
6 Shi, Z-D. *et al.* (2004) Synthesis of a 5-methylindolyl-containig macrocycle that displays ultrapotent Grb2 SH2 domain-binding affinity. *J. Med. Chem.* 47, 788–791

Luca Costantino
Costantino.luca@unimo.it

Biology

Microbiology

Respiratory chain inhibition and pentamidine: a new drug combination against leishmaniasis?

Chemotherapy constitutes the main tool for control of leishmaniasis, but antileishmanial drugs are rare, expensive and toxic, and their mechanisms of action are elusive. Mehta and Shaha now show that concomitent administration of respiratory chain complex II inhibitors with pentamidine increases the drug cytotoxicity against promastigotes of *Leishmania donovani* [1].

The authors first observed that inhibition of the respiratory complexes II and III by TTFA and antimycin A, respectively, resulted in the dissipation of the mitochodrial membrane potential. On the contrary, rotenone, an inhibitor of respiratory complex I induced a mitochondrial hyperpolarization. These changes in Dym caused, as expected, a reduction of the cell viability, but more interestingly this loss was attributed to apoptosis as cells showed DNA fragmentation and externalization of phosphatidyl serine.

In a second step the authors tried to understand by which mechanism the Dym alteration occured. They observed that inhibition of complexes II and III resulted in the generation of reactive oxygen species (ROS) and in an increase of the intracellular calcium concentration. Because ROS increase was not blocked by calcium sequestration (EGTA) and an ROS scavenger inhibited the increase in intracellular calcium, the authors concluded that ROS generation was responsible for the calcium increase.

Pentamidine was known to accumulate in the mitochodria causing a loss of Dym . The combined use of pentamidine and TTFA led to a fourfold increase in intracellular calcium and an increase in cell

1359-6446/04/$ – see front matter PII: S1359-6446(04)03060-0

death. However, the mechanism of calcium increase was different because EGTA did not block it.

The authors propose that inhibition of complex II during pentamidine treatment might provide the biochemical basis for the increased efficacy of pentamidine. It would be interesting to identify from which cell compartment calcium originates and to test the efficacy of pentamidine on acidocalcisome-deficient strains or the TTFA action on pentamidine-resistant strains.

1 Mehta, A. and Shaha, C. (2004) Apoptotic Death in *Leishmania donovani* Promastigotes in Response to Respiratory Chain Inhibition: complex II inhibition results in increased pentamidine cytotoxicity. *J. Biol. Chem.* 279, 11798–11813

Norbert Bakalara
bakalara@u-bordeaux2.fr

One dose of whole mycobacterial cell vaccine will protect if the right adjuvant is used

Recent efforts to develop a new generation of tuberculosis (TB) vaccines are described in the April issue of *Vaccine*. Preliminary evidence supports the growing potential of developing novel and efficacious killed whole-cell mycobacterial vaccines by using new style adjuvants. Today, the only vaccine available for prevention of tuberculosis in humans is a live attenuated vaccine, bacille Calmette-Guerin (BCG). Low production costs, safety and efficacy have made it a long-standing success, but with growing concern about its efficacy and use in immunocompromized individuals there have been increased efforts to investigate new generation of TB vaccines. With recent evidence suggesting that protein subunit vaccines in appropriate adjuvant can protect mice, immunity to numerous antigens is likely to give full protection.

With this in mind, Chambers *et al.* [2] describe formalin-killed whole-cell mycobacterial preparations in selected liposome adjuvants to vaccinate guinea pigs. The adjuvants chosen were recognized to induce Th1 responses while possessing adjuvant properties. All Novasome™ liposomes were designed to have a net negative charge for a more rapid removal from the circulation, to increase the ability to localize to specific organs and become effectively trapped in the lungs. The correct choice of adjuvant is essential for successful immunization because some preferentially induce type 1 or type 2 immune responses.

The vaccines were administered to guinea pigs resulting in protection of animals against lethal challenge with low dose aerosol of viable *Mycobacterium bovis*. The group successfully discuss their conclusions and propose further studies that need to be addressed to fully support the continuation of this preliminary study.

2 Chambers, M.A. *et al.* (2004) A single dose of killed *Mycobacterium bovis* BCG in a novel class of adjuvant (Novasome™) protects guinea pigs from lethal tuberculosis. *Vaccine* 22, 1063–1071

Silvana Bettiol
silvana_bettiol@yahoo.com

Cancer

PPARγ: tumour promoter or tumour suppressor?

The ligand-sensitive transcription factor peroxisome proliferator-activated receptor γ (PPARγ) is overexpressed in several cancers, and treatment of tumour cell lines with PPARγ agonists results in growth arrest and differentiation. Hence, PPARγ represents a potential therapeutic target.

To determine the effect of PPARγ in breast cancer, Saez and colleagues generated a mammary gland-specific transgenic mouse model, expressing a ligand-independent form of the protein [3]. Expression of this constitutively active transgene had no effect on normal development or function. However, when the mice were crossed with a strain prone to mammary gland tumours, the bigenic animals developed tumours at an accelerated rate, suggesting that in the presence of a cancer-initiating event PPARγ acts as a tumour promoter.

The Wnt signalling pathway plays a causal role in breast cancer. Using the developing zebrafish embryo as a model, the authors were able to demonstrate that PPARγ activation results in enhanced Wnt signaling. Injection of constitutively active PPARγ, or wild-type PPARγ stimulated with the agonist rosiglitazone, produced a phenotype indistinguishable from that observed following injection with Wnt8c. In addition, activation of PPARγ resulted in the upregulation of known Wnt target genes.

PPARγ agonists are currently being evaluated as chemotherapeutic agents. However, the findings of this study suggest that PPARγ activation can actually result in context-dependent tumour promotion. In addition, PPARγ agonists are widely used to manage type-2 diabetes. It is therefore crucial that the long-term effects of drug administration are rigorously investigated.

3 Saez, E. *et al.* (2004) PPARγ signaling exacerbates mammary gland tumor development. *Genes Dev.* 18, 528–540

Vicky Heath
vjh2r@udcf.gla.ac.uk

Neuroscience

Bradykinin receptor antagonism is poised to slow pain development

Bradykinin is an autacoid peptide consisting of nine amino acids that, among other functions, plays an important role in the development and maintenance of acute and chronic pain following inflammation and nerve damage. Bradykinin binds to two distinct G-protein-coupled receptors, denoted bradykinin B_1 and bradykinin B_2, based on their unique pharmacological properties and patterns of expression.

The B_2 receptor is constitutively expressed in nearly all cell types and is thought to play a role in the acute phase of the inflammatory pain response. By contrast, B_1 receptors, found in sensory

nerve ganglia, which convey information about pain from the periphery to the spinal cord and brain as well as in central and peripheral endings of sensory neurons, are induced during infection and tissue injury. B_1 expression is induced by cytokines that become liberated after injury, and is thought to play a role in the chronic, more long-term, phase of pain. B_1 receptors elicit persistent neuronal firing responses, which are not limited by desensitization, receptor internalization, or ligand dissociation. Further amplification of painful stimuli occurs upon long-term agonist exposure whereby the B_1 receptor is upregulated. Activation of B_1 receptors on other proliferative cell types appears to be responsible for releasing mediators that sensitize or activate nociceptors.

Very recently, a new high-affinity, subtype-selective, non-peptide antagonist of the bradykinin B_1 receptor has been developed and characterized [4]. This compound, termed SSR240612, successfully inhibits B1 receptor binding of bradykinin metabolites, inhibited desArg^9-BK and capsaicin paw and ear edema in mice, and tissue destruction and neutrophil accumulation in rat intestine after ischemia/reperfusion injury. SSR240612 also inhibited thermal hyperalgesia by UV irradiation, the late phase of nociceptive response to formalin administration in rats, as well as the development of neuropathic thermal pain by sciatic nerve constriction injury in rat.

The potentially undesirable influence of SSR240612 in nullifying the protective effects of B_1 receptors in CNS inflammatory diseases such as multiple sclerosis, by reducing T-lymphocyte infiltration into the brain, must be closely examined before its value can be completely realized, and before moving towards an effective treatment for chronic pain, but B_1 receptor antagonists appear poised to have a meaningful impact.

4 Gougat, J. *et al.* (2004) SSR240612, a new non-peptide antagonist of the bradykinin B1 receptor. Biochemical and pharmacological characterization. *J. Pharmacol. Exp. Ther.* DOI: 10.1124/jpet.103.059527 (E-pub ahead of print; http://www.jpet.org)

Bryan C. Hains
bryan.hains@yale.edu

Genomics and proteomics

A new approach for drug target identification

The finding of new drugs requires identification of the target in order to understand the mode-of-action of the chemicals. However, this step is often a limiting factor. More than 40% of yeast genes share conserved sequence with at least one human gene, and the mode-of-action of a drug could be conserved between yeast and mammalian cells, therefore the authors of a recent paper [6] proposed a pathway/target identification procedure by using a drug-induced haploinsufficiency in yeast.

This approach is divided into five stages and illustrated for the target identification of dihydromotuporamineC(dhMotC), which is known to inhibit invasion of human carcinoma cell lines.

The first stage found that 80 μM of dhMotC totally inhibited yeast growth. In a second stage, 5000+ heterozygous diploid deletion strains were screened for their sensitivity to 60 μM dhMotC and 6 essential and 15 non-essential genes were found. To confirm this sensitivity, the authors quantitatively evaluated the sensitivity of the 21 strains by an integrative growth curve difference analysis. Two strains (LCB1, TSC10) were more sensitive to dhMotC. Both of them were deleted for genes involved in the sphingolipid biosynthesis.

The fourth stage consisted in confirming in yeast the interference of dhMotC with the sphingolipid metabolism. Exogenous addition of DHS (dihydrosphingosine) to the two selected heterozygous diploid deletion strains suppressed the growth inhibition, and rescued the organization and depolarization of the actin cytoskeleton, induced by dhMotC.

Finally, the authors confirmed, in wild-type cells, that dhMotC acts against the sphingolipid metabolism by reducing the cellular ceramide levels. They also showed that 50 nM of C6-ceramide increased the survival of cells exposed to dhMotC by 4–5 times.

The authors mentioned two restrictions for their system: (1) the screen did not identify all the genes of the sphingolipid metabolism and (2) the extent of protection induced by C6- ceramide in human cells was not as high as observed in yeast. They suggest that the functional gene redundancy and the fact the C6-ceramide harbors a cytotoxic effect might explain these restrictions.

6 Baetz, K. *et al.* (2004) Yeast genome-wide drug-induced haploinsufficiency screen to determine drug mode of action. *Proc. Natl. Acad. Sci. U. S. A.* 101, 4525–4530

Norbert Bakalara
bakalara@u-bordeaux2.fr

IAPs halt caspase death cascade following ischemic preconditioning

In recent years, neuroscientists have developed a growing appreciation in the role of apoptosis in ischemia-induced cell death. The CA1 subfield of the hippocampus, a brain region that is involved in spatial and temporal information processing, is particularly vulnerable to ischemic insults. Ischemic tolerance or preconditioning is a phenomenon in which a brief period of lethal ischemia confers robust protection to CA1 neurons to a subsequent, otherwise lethal, ischemic insult. However, it is not known whether or not apoptotic signalling proteins contribute to ischemic tolerance.

Tanaka *et al*. investigated the molecular mechanisms underlying ischemic tolerance by producing transient global cerebral ischemia in rats by the four-vessel occlusion (4 minutes preconditioning; 48 h recovery followed by 10 minutes global ischemia) procedure. Toluidine blue-stained coronal brain sections revealed that ischemic preconditioning provided neuroprotection against ischemia-induced cell death in the hippocampal CA1. Western immunoblotting, immunohistochemistry and *in vitro* enzymatic assays indicated that global ischemia triggers the activation of the major components of apoptosis, the

pro-apoptotic caspases-3 and -9. But in contrast to persistent caspase-3 activity in 'single ischemic' neurons, preconditioning elicited marked, but only transient, activation of caspase-3.

The inhibitors of apoptosis (IAPs) are endogenous caspase inhibitors. The protein expression level of a member of the IAP family, cIAP-2, was upregulated following global ischemia, regardless of preconditioning. However, an IAP antagonist, Smac/DIABLO, was no longer released from the mitochondria in preconditioned neurons. Together, these results suggest that preconditioning enable CA1 neurons to survive by maintaining mitochondrial membrane integrity, thereby preventing Smac-mediated inhibition of IAPs and allowing IAPs to inhibit caspase-dependent apoptosis.

By identifying the players involved in ischemic tolerance, these findings provided valuable insight into the molecular mechanisms for the prevention of neuronal death associated with stroke and other neurodegenerative diseases. Interestingly, in addition to their anti-caspase activity, previous studies demonstrated that IAPs also function as negative regulators of Smac via their ubiquitin ligase activity. Therefore, a therapeutic strategy utilizing IAPs to block intrinsic apoptotic pathways might be suitable in ameliorating the outcome of neuronal injuries.

5 Tanaka, H. *et al.* (2004) Ischemic preconditioning: neuronal survival in the face of caspase-3 activation. *J. Neurosci.* 24, 2750–2759

Herman H. Cheung
herman@mgcheo.med.uottawa.ca

Business

Collaborations

Inpharmatica announces ADME technology agreement with GSK

Inpharmatica (http://www.inpharmatica.co.uk/), the selective drug discovery company, has announced that it has entered into an agreement with pharmaceutical company, GlaxoSmithKline (GSK; http://www.gsk.com/) to access its ADME technologies. Under the agreement, Inpharmatica will apply its proprietary *in silico* ADME technology to an undisclosed number of GSK's drug discovery programmes in the UK and Europe.

This is the fourth ADME deal to be announced by Inpharmatica in the past six months, providing further validation of the company's expertise and industry-leading technology in this important area in drug discovery. Mike Tarbit, Senior VP of pre-clinical R & D, at Inpharmatica, said; 'we are delighted with the ongoing success of our ADME business and the calibre of partners we are attracting.'
Inpharmatica's lead discovery programme is focused on 16 novel nuclear receptors, representing a druggable protein family of high therapeutic interest. The company's chemogenomics technology platform uses one of the largest computer farms in the world.

NIH and FDA launch new human gene transfer research data system

The National Institutes of Health (NIH) and the Food and Drug Administration (FDA) announced today that they have launched a new genetic modification clinical research information system, GeMCRIS (http://www.gemcris.od.nih.gov/) – a web-accessible database on human gene transfer (gene therapy). GeMCRIS, developed collaboratively by the two agencies, is a unique public information resource, as well as an important new electronic tool to facilitate the reporting and analysis of adverse events on these trials. The new system will provide information to the public directly and will improve the government's ability to monitor adverse events in gene therapy.

NIH Director Elias A. Zerhouni, said, 'GeMCRIS is an important achievement and a unique resource for scientists, patients, and the public. GeMCRIS will help advance gene therapy, while allowing NIH, FDA, and the research community to maintain appropriate oversight.'

Acting FDA Commissioner Lester M. Crawford, emphasized that 'the development of GeMCRIS illustrates the government's commitment to addressing public and patient concerns about safety while advancing gene therapy. Providing accurate and complete information about ongoing gene therapy studies is the best way to achieve this goal.'

Protein Mechanics and Aventis

Protein Mechanics (http://www.proteinmechanics.com/) and Aventis (http://www.aventis.com/) have entered into an agreement to advance the identification, discovery and validation of highly selective, orally available matrix metalloprotease (MMP) inhibitors, a promising new class of drugs for treatment of a broad array of pathologies involving the extracellular matrix.

This protein family presents significant selectivity challenges when approached with conventional methods. Protein Mechanics will utilize its Imagiro® predictive simulation technology to provide Aventis with insights into important aspects of specific target-ligand interaction.

Ken Haas, President and CEO of Protein Mechanics, Inc. said 'Aventis has a strong record of commitment and accomplishment in the development of novel therapeutics for important disease areas, so we are extremely gratified that it has chosen Protein Mechanics as a partner in this effort.'

Business was written by
Matthew Thorne

People

Retirement

USP announces retirement of Joseph G. Valentino

The United States Pharmacopeia (USP; http://www.usp.org/) have announced that Joseph Valentino., Senior Vice President and General Legal Counsel at USP, will be retiring after more than 35 years.

Valentino currently directs USP's legal activities. He is also secretary to the USP Board of Trustees and the USP Convention.

1359-6446/04/$ – see front matter PII: S1359-6446(04)03080-6