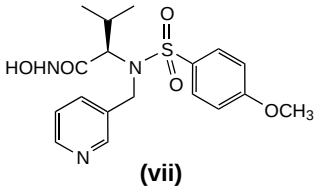
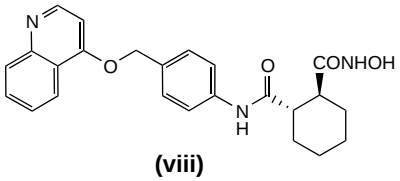
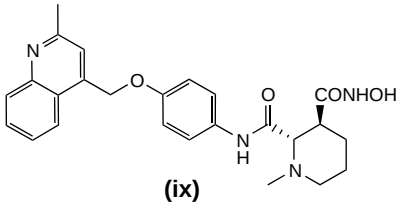
A group from Bristol–Myers Squibb (http://www.bms.com) used the Ciba–Geigy (http://www.ciba.com) sulfonamide CGS27023A (**vii**) and its cocrystal structure bound to MMP-3 to rationally design a series of cyclic succinate-based hydroxamic acid inhibitors of TACE, as exemplified by compound **viii** [8]. This compound shows good potency (IC_{50} of 8 nM) and high selectivity, possessing a K_i >2 μM for MMP-1, MMP-2, MMP-9 and all members of the related metalloprotease family. However, compound **viii** is an ineffective inhibitor (IC_{50} of >50 μM) of TNF-α release in a whole blood assay (WBA). This result was thought to be a consequence of the lipophilicity of the cyclohexyl ring giving rise to high plasma-protein binding. A nitrogen atom was introduced into the cyclohexyl ring to generate a new series of 3,4-piperidine carboxamide derivatives [9]. The introduction of a basic nitrogen atom



(vii)



(viii)



(ix)

greatly improved activity in the WBA but compounds often showed poor permeability in the Caco-2 assay. Walking the nitrogen around the ring eventually led to compound **ix**, which possesses an IC_{50} of 6.2 nM for TACE, maintains selectivity over related MMPs and has an IC_{50} of 20 nM in the WBA. The molecule demonstrated reasonable Caco-2 permeability and an oral bioavailability of 43% in dog.

8 Xue, C-B. *et al.* (2003) Rational design, synthesis and structure–activity relationships of a cyclic succinate series of TNF-α converting enzyme inhibitors. Part 1: Lead identification. *Biorg. Med. Chem. Lett.* 13, 4293–4297

9 Xue, C-B. *et al.* (2003) Rational design, synthesis and structure–activity relationships of a cyclic succinate series of TNF-α converting enzyme inhibitors. Part 2: Lead optimization. *Biorg. Med. Chem. Lett.* 13, 4299–4304

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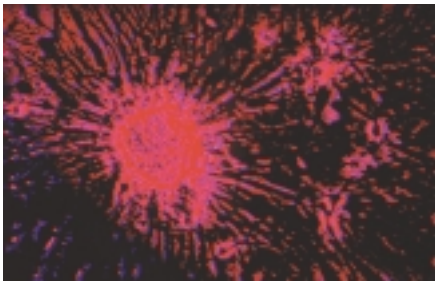
Biology

Cancer

New therapeutic potential for NSAIDs



Non- steroidal anti-inflammatory drugs (NSAIDs) inhibit the enzymatic activity of cyclooxygenase (COX), a protein catalyzing a key step in the synthesis of prostaglandins and often involved in the development and progression of colorectal cancer. Two isoforms have been identified so far. COX-1 is widely expressed and constitutively active, whereas COX-2 was found to be involved in inflammatory processes. Several Rho GTPases control COX-2 expression via regulation of transcription factor activity.

Rho GTPase deregulation has been reported for several human tumours, including breast, colon, pancreas, and head and neck squamous carcinomas. In a recent study [1], Benitah *et al.* analyzed the effects of two NSAIDs (Sulindac and NS-398) on COX-2 expression.

Constitutively active (QL) RhoA, Rac1 and cdc42 were shown to induce COX-2 expression in transformed NIH3T3, HT29 and MDCK cells. Among the various effectors of Rho GTPases, ROCK kinases were identified as mediators of COX-2 expression via NF-κB, which has a putative binding site in the COX-2 promotor region.

Treatment of RhoA-, Rac1- and cdc42-transformed cells with either Sulindac or NS-398 resulted in inhibited growth and proliferation and led to complete loss of COX-2 expression. NSAIDs were shown to inhibit NF-κB activation, as well as translocation to the nucleus. The capacity of NS-398 to inhibit growth *in vivo* was determined in mice transfected with MDCK-RhoAQL cells. Upon treatment with NSAIDs, a decrease in size of RhoAQL induced tumours was observed, compared to untreated mice.

NSAIDs were shown to limit Rho induced signaling by inhibiting activity of NF-κB. The results suggest that treatment of Rho disregulated tumours with specific NF-κB- inhibitors or NSAIDs like sulindac and NS-398 might offer a potential new avenue for antitumour therapy.

1 Aznar Benitah, S. *et al.* (2003) ROCK and nuclear factor- kappaB- dependent activation of cyclooxygenase-2 by Rho GTPases: effects on tumor growth and therapeutic consequences. *Mol. Biol. Cell* 14, 3041–3054

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VCP: a new role at the end of mitosis

VCP/p97/Cdc48 is a ubiquitous ATPase related to N-ethylmaleimide sensitive factor (NSF) and proteasomal ATPases. It is involved in different cellular activities, ranging from homotypic membrane function, ER-associated degradation, apoptosis, neurodegeneration and cancer

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metastasis. The common feature of these different VCP functions might be its ability to extract proteins from multisubunit complexes, the specificity being afforded via its interaction with different adaptor proteins, such as p47, Ufd1/Npl4, SVIP.

Cao *et al.* [2] now present compelling evidence for yet another VCP function: spindle disassembly at the end of mitosis. Addition of a dominant-negative VCP mutant to the well established *in vitro* model of spindle formation and disassembly around decondensed sperm nuclei in *Xenopus* egg extracts did not interfere with spindle formation. However, it prevented spindle disassembly upon the decrease of Cdc2 activity to interphase levels. Similar effects were achieved by immunodepletion of the less abundant Ufd1/Npl4 adaptor complex from the extracts. The VCP–Ufd1–Npl4 complex interacts with several spindle-assembly factors at the end of mitosis, possibly recruiting them for proteasome-dependent degradation.

Another study [3] reports that depletion of VCP from HeLa cells by RNA interference induces block in mitosis with multiple spindle abnormalities. The findings of Cao *et al.* explain this phenotype, implying that the role of VCP in spindle disassembly is probably similar in all eukaryotes. The role of VCP in spindle function might also explain the role of VCP in cancer. VCP could therefore be a new potential target for antimitotic drugs.

2 Cao, K. *et al.* (2003) The AAA-ATPase Cdc48/p97 regulates spindle disassembly at the end of mitosis. *Cell* 115, 355–367

3 Wójcik, C. *et al.* (2003) RNA interference of valosin-containing protein (VCP/p97) reveals multiple cellular roles linked to ubiquitin/proteasome-dependent proteolysis. *J. Cell Sci.* 117, 281–292

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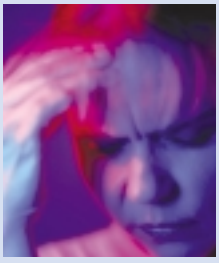
Neuroscience

Pain in the brain: opioids regulate responses to emotional pain



Exciting new work shows that a sustained state of sadness is associated with a decrease in endogenous opioid neurotransmission in anterior cingulate and subcortical limbic brain regions in healthy women [8]. The findings reveal that neuroimaging techniques, such as positron emission tomography (PET), can be used to measure dynamic changes in neurotransmission during the generation of affective states in humans, which could eventually lead to effective treatment of disorders such as depression.

Zubieta *et al.* showed that binding of the radiotracer [^{11}C] carfentanil to mu-opioid receptors in the brain was increased during sad mood states compared with neutral state, indicating that sad mood is accompanied by a decrease in endogenous mu-opioid neurotransmission.

The sadness-induced deactivation of opioid neurotransmission was localized to the rostral anterior cingulate, inferior temporal cortex, amygdala and ventral pallidum; regions previously implicated in the processing of emotional information.

These findings reinforce the role of specific brain regions in normal emotional processing, and are interesting in the light of reported abnormalities in neural activity in the rostral anterior cingulate and amygdala in patients with major depression. This study suggests that affective responses in these brain regions are regulated by opioid neurotransmission, with a decrease in opioid release appearing to have a permissive effect on the experience of sad mood.

Zubieta *et al.* describe some intriguing data linking abnormalities in mu-opioid neurotransmission with anxiety and suicide. More such studies should elucidate the complex interactions between the neurotransmitter systems that govern our responses to emotional stimuli and regulate our mood states.

8 Zubieta, J.K. *et al.* (2003) Regulation of human affective responses by anterior cingulate and limbic mu-opioid neurotransmission. *Arch. Gen. Psychiat.* 60, 1145–1153

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Targets and Mechanisms

Antimalarial mechanisms of the cinchona alkaloids

Quinine is the most abundant of the cinchona alkaloids, and has thus been the most important as an antimalarial drug. A recent publication by Warhurst *et al.* on the cinchona alkaloids provides valuable insight into their mechanism of antimalarial action [4].

Recent structure–activity studies of the related aminoquinolines have highlighted the likely importance of two factors in their activity against *Plasmodium falciparum*: the pKa values of these weak base compounds, and their ability to inhibit the conversion of haematin to haemozoin. There is some evidence that quinine (Q) and quinidine (QD) might act in a similar way.

This study has provided evidence that this is indeed so. QD is more active against malaria parasites than Q, whereas the epi analogues (EQ and EQD) essentially inactive. Thus, Warhurst *et al.* have investigated all cinchona alkaloids; they measured the pKa values, log P values and β-haematin inhibitory activity values of all compounds and compared them with reported biological activities. The results demonstrate several key differences between the active erythro compounds (Q, QD) and the inactive threo compounds (EQ and EQD). The result is that these compounds will enter the FV of the parasite much more slowly by passive diffusion than the erythro analogues.

In addition, the study confirms that EQ and EQD do not strongly inhibit β-haematin formation and molecular modelling further indicates that these differences can be traced to different optimal orientations of the quinuclidine side-chains of these compounds and their restricted ability to rotate.

The study provides an interesting starting point for more detailed and rigorous investigations of the origins of the biological activities of these compounds.

4 Warhurst, D.C. *et al.* (2003) The relationship of physico-chemical properties and structure to the differential antiplasmodial activity of the cinchona alkaloids. *Malaria J.* 2, 26–40

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Immunology

IL-4 crucial for Th-1 type of immune response against *Leishmania*

It has been shown recently [9] that induction of a protective immune response against *Leishmania donovani* with a single protein requires interaction of the antigen with a natural antibody, which leads to triggering of complement-dependent pathways of interleukin (IL) 4 production. Importantly, IL-4 production is crucial for the generation of protective CD8+ T lymphocytes. This pathway has not been described previously and has implications for immunology in general and vaccination in particular.

Stäger *et al.* [9] showed that protective CD8+ T-cells could be induced by injection of a candidate vaccine antigen against *Leishmania* (hydrophilic acylated surface protein B-1; HASPB-1) without adjuvant. IL-4 knockout mice could not be vaccinated and, in contrast to wildtype, HASPB-1-specific CD8+ T cells were not increased after vaccination. This points to a link between IL-4 production and cell-mediated immunity through CD8+ cytotoxic T cells.

The study also provided evidence that, upon injection of HASPB-1 antigen, the primary source of IL-4 production were $CD4^{+}CD11c^{lo}$ mononuclear phagocytes. Interestingly, IL-4 production could not be induced in SCID-mice, but this could be restored through transfer of serum from normal mice, which suggests that natural antibodies had a role in the induction of IL-4 secretion, and that there should be natural antibodies that specifically recognise the HASPB-1 antigen.

These results suggest that, upon injection of the HASPB-1 antigen, it interacts with a natural antibody to form an immune complex, which interacts with $CD4^{+}CD11c^{lo}$ mononuclear phagocytes to trigger IL-4 secretion. In addition, complement is essential for this activation of IL-4-producing cells.

These findings show that dissection of the immune system in humoral and cell-mediated, or Th-1 type versus Th-2 type components, is misleading and that typical humoral components have a crucial role in the development of Th-1 type of cell-mediated immune reaction patterns through induction of IL-4.

9 Stäger, S. *et al.* (2003) Natural antibodies and complement are endogenous adjuvants for vaccine-induced cd8+ T-cell responses. *Nat. Med.* 9, 1287–1292

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Chloroquine resistance caused by drug efflux

Evidence to date provides compelling support for the *Plasmodium falciparum* chloroquine resistance transporter (PfCRT) being responsible for chloroquine resistance. However, our knowledge of the cellular mechanisms of chloroquine resistance remains uncertain. Now, a study by Michael Lanzer and co-workers has provided new evidence for an efflux mechanism [5].

The study confirmed that ^{3}H-labelled chloroquine is taken up in both chloroquine-sensitive (CQS) and chloroquine-resistant (CQR) parasitized red blood cells (pRBCs) in glucose-free medium. The uptake is similar in CQS and CQR pRBCs, as is the concentration of intracellular ATP upon addition of glucose. However, when glucose is added, the CQS pRBCs take up considerably more chloroquine, while the chloroquine concentration in CQR pRBCs is rapidly reduced.

What is novel, are the trans stimulation experiments where pRBCs are pre-loaded with unlabelled CQ: when labelled CQ is added to CQS parasites, the uptake of CQ into the cells is reduced in a hyperbolic concentration-dependent manner. However, when the experiment is conducted with CQR pRBCs there is an increase in uptake of labelled CQ when unlabelled CQ is present at low concentration. A decreased uptake of labelled CQ is seen only in the presence of higher concentrations of unlabelled CQ.

These observations indicate that the unlabelled CQ directly competes with the labelled drug for binding to the CQR machinery, which is most easily explained by an efflux mechanism.

5 Sanchez, C.P. *et al.* (2003) Trans stimulation provides evidence for a drug efflux carrier as the mechanism of chloroquine resistance in Plasmodium. *Biochem.* 42, 9383–9394

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Miscellaneous

Commensals can modulate the ability of a pathogen to invade cells

Little is known about how commensal bacteria present in the intestinal lumen affect the pathogenic capabilities of gastrointestinal pathogens. A recent study by Lyczak [6] presents evidence indicating that commensal bacteria can serve to increase the invasion of intestinal epithelial cells by *Salmonella typhi*.

First, Lyczak showed that water extracts made from commensal bacteria are able to trigger redistribution of the cystic fibrosis transmembrane conductance receptor (CFTR) to the plasma membrane. The CFTR protein is the receptor used by *S. typhi* for binding and invasion of intestinal epithelium. Next, using fluorescence microscopy, Lyczak showed that the CFTR modulation by commensal bacterial extracts is correlated with enhanced *S. typhi* binding to the epithelial cell surface. Finally, Lyczak demonstrated that commensal extracts with the greatest ability to enhance CFTR redistribution were able to significantly enhance *S. typhi* invasion of epithelial cells in a ligated intestinal loop model. As a control, Lyczak also showed that the invasion capabilities of bacteria that do not invade cells via the CFTR were not affected by the commensal extracts.

The results of this study show that some kinds of commensal bacteria have the potential to modulate the susceptibility of the human host to some pathogens. This is important, because the population of normal flora in a given individual is not static and can be affected by changes over the course of a lifetime.

6 Lyczak, J.B. (2003) Commensal bacteria increase invasion of intestinal epithelium by *Salmonella enterica* Serovar Typhi. *Infect. Immun.* 71, 6610–6614

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Staphylococcus aureus gets a helping *van*(d) from *Enterococcus faecalis*

One of the last lines of treatment against *Staphylococcus aureus*, including the methicillin-resistant strains (MRSA), has been vancomycin. Strains with decreased susceptibility to vancomycin (VISA) have been isolated previously, but recently a vancomycin-resistant strain (VRSA) was isolated from a dialysis patient in Michigan. Vancomycin-resistant *Enterococcus faecalis* and *E. faecium* (VRE) has been a major problem for the last decade. The resistance genes in VRE are carried on the mobile genetic element, transposon Tn*1546*, with the potential for interspecies transfer to, for instance *S. aureus*.

Weigel *et al.* [7] isolated plasmids from the Michigan VRSA strain and two co-isolates from the same patient, a VRE and a vancomycin-susceptible MRSA. Both VRSA and VRE plasmids contained Tn*1546*, while the MRSA did not, suggesting that the MRSA acquired the transposon from the VRE, thus generating a VRSA. The VRSA and VRE plasmids were shown to be conjugative plasmids that can be transferred *in vitro* to *S. aureus* and *E. faecalis*, respectively.

When the complete sequence of the VRSA plasmid was determined, it revealed a composite structure with homologies to several multidrug resistance plasmids and conjugative plasmids. The integrated transposon encoding the VR gene cluster was identical to the prototype Tn*1546*.

In addition to the Tn*1546*, genes encoding putative resistances against several other antibiotics and disinfectants were identified.

This study convincingly shows that VRSA can arise from inter-species conjugation and/or transduction events of mobile genetic elements, most likely originating from co-infecting vancomycin-resistant enterococci, but also raises serious concerns about continuous intra-species dissemination among *S. aureus.*

7 Weigel, L.M. (2003) Genetic analysis of a high-level vancomycin-resistant isolate of *Staphylococcus aureus*. *Science* 302, 1569–1571

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Business

Announcements

UniProt is launched

The EMBL-European Bioinformatics Institute (EBI), the Swiss Institute of Bioinformatics (SIB) and Georgetown University Medical Center's Protein Information Resource (PIR), have announced the launch of UniProt, a new universal protein resource that will be the most comprehensive catalogue of information on proteins.

This venture was made possible by funding from the US National Institutes of Health, totalling US$15 million over three years, with the National Human Genome Research Institute (NHGRI) as the primary funding body.

Peter Good, the NHGRI programme director in charge of the UniProt project, said: 'The UniProt databases will be a critical resource for investigators trying to unlock the secrets in genome sequences, both to understand biology and to translate basic research into improvements in healthcare.'

Rolf Apweiler, UniProt's Principal Investigator, explained, 'UniProt's structure resembles that of a wedding cake. Each tier of the cake represents a different database, optimized for different uses.'

The next layer of the 'wedding cake', and the centrepiece of activities for the three institutes – collectively known as the UniProt Consortium – is the UniProt Knowledgebase, which is unified from Swiss-Prot, TrEMBL and PIR-PSD. 'This is the place to go if you want to know everything there is to know about a specific protein,' explains Maria-Jesus Martin, Sequence Coordinator for EBI.

UniProt can be accessed at http://www.uniprot.org.

Collaborations

Entelos expands research with Johnson & Johnson

Entelos (http://www.entelos.com) has announced its expansion of its research collaboration with Johnson & Johnson Pharmaceutical Research and Development (J&JPRD; http://www.jnj.com), to include target validation, lead optimization and clinical development in obesity.

In addition, J&JPRD sister company – McNeil Nutritionals – will join the collaboration. This announcement coincides with the first annual meeting of the Diabetes Research Forum, which is a research effort between Entelos, the American Diabetes Association and the pharma industry, to advance research in the treatment of type 2 diabetes.

James Karis, President and CEO of Entelos, said that he was pleased that J&JPRD had expanded their collaboration to include obesity and that McNeil Nutritionals has joined the collaboration.

Entelos employs disease level system biology technologies to identify and validate targets, biomarkers and compounds for human efficacy.

Cellzome in collaboration with Bayer Healthcare

Cellzome (http://www.cellzome.com) have announced a collaboration with Bayer Healthcare (http://www.bayer.com), where Cellzome's drug proteomics platform will be leveraged to profile Bayer's lead compounds.

Cellzome's chemical proteomics approach will be used to identify the protein interaction profiles of several lead compounds from a variety of therapeutic areas, facilitating drug development at Bayer.

David Brown, Chief Executive at Cellzome, commented: '… [we] believe this is the first step towards a long-term strategic relationship between the companies.'

Cellzome is a drug discovery company building an R&D pipeline in chronic diseases, with a primary focus on Alzheimer's disease, whose combination of chemical proteomics and pathway expansion focuses on the interface between validated disease pathways, tractable medicinal chemistry and druggable targets.

Business was written by *Joanne Clough*

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