The Newsletter for the Society for Medicines Research

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SMR Meeting on Angiogenesis

by Irene François

On 9 July 1999, the Society held a one-day meeting on Angiogenesis at the National Heart and Lung Institute, London. The speakers covered many aspects of this fundamental process, both in normal development and in a variety of angiogenic-dependent diseases.

The meeting opened with an overview by **Professor Helmut Augustin (University of Gottingen, Germany)**. Three processes involved in vascular morphogenesis were defined.

- Angiogenesis: the sprouting of new capillaries from pre-existing ones.
- Vasculogenesis: the *in-situ* differentiation of a primary capillary plexus from haemangioblastic stem cells; and
- Intusseption: the branching of a capillary by longitudinal splitting.

In adults the only organ site with physiological angiogenesis is the female reproductive system. During ovulation there is dramatic growth of the *corpus luteum* due to an increase in angiogenesis. At the end of the cycle there is a regression in sprouting and the growth of blood vessels. Professor Augustin's research team have compared the cyclic angiogenic processes in the ovarian vasculature that regulate the 'life-cycle' of the transient *corpus luteum* with the pathological angiogenesis that is associated with tumour growth.

Angiogenesis is under multiple control and there are now between 10 and 20 different factors known to elicit neovascularisation. Hypoxia and acidosis in the microenvironment of a tumour stimulate specific angiogenic growth factors such as VEGFs-A, B, C, D and E and PIGF. Local angiogenic stimulators of pleiotropic nature include bFGF, aFGF, TGFα, TGFβ, and PD-ECGF. Autocrine and paracrine processes in angiogenesis are associated with endothelial cell activity involving cytokines, adhesion molcules and chemokines. Secondary enhancement leads to recruitment of monocytes and leukocytes while the capillary organisation involves angiopoietins.

There are positive and negative regulators of angiogenesis. Positive regulators include VEGF, PIGF, TGFs, angiogenin, IL-8, HGF, GCSF and PDGF. The endogenous negative regulators of angiogenesis that have been identified include thrombospondin, angiostatin and glioma-derived angiogenesis inhibitor factor. The angiopoietins (Ang1, 2, 3, 4) are antagonistic and control the maturation of new vessels. The Ang2 to Ang1 ratio is important. The ratio is 1: 1 in the the resting and new growth state of

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SMR Chairman Irene François (right) at the Protein Kinase Symposium dinner

Viewpoint

Investing in the Future

by Julie Holder & David Nash

This article is the first in our viewpoint series, introduced last issue. The authors will receive two bottles of champagne for their efforts.

In order for the pharmaceutical industry to flourish in the 21st century, the supply of high-calibre scientific personnel needs to be assured. The industry recognises that this is predicated upon stimulating a greater interest in science at all levels of education, and in particular within primary and secondary schools.

In common with other major pharmaceutical companies, SmithKline Beecham (SB) has established school-liaison committees at both the primary and the secondary levels in order to support local schools by liaison with a key science contact within these schools. In addition, SB has appointed a school-liaison co-ordinator (SLC) who is an active science teacher seconded from a local secondary school.

The responsibilities of the SLC are to co-ordinate effective internal and external interactions and strengthen existing scientific links with local schools. SB personnel are encouraged to take an active interest in school liaison and take part in school open days, industry weeks and science fairs.

Science is a core subject within the National Curriculum, and is taught from the age of five. The primary committee was set up to assist local schools to deliver an interesting science curriculum. Each year the committee organises two Open Days for local schools at which 200 children learn about the drug-discovery process with an emphasis on demonstrations and hands-on activities. The primary group hosts an annual series of lectures modelled on the Royal Institution Lectures for 10/11-year-olds. In 1998, the theme was 'Science and Energy' and

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Protein Kinases: Therapeutic Opportunities

by the SMR Committee

The latest trends in protein kinase and phosphatase research were described in a one-day symposium 'Protein Kinases: Therapeutic Opportunities' organised by the Society for Medicines Research. The meeting was held on 25 March 1999 at Pfizer's research complex in Sandwich, Kent. Seven speakers from academia and industry provided a clear view of the role of kinases and phosphatases in cell signalling and disease, followed by discussion on the therapeutic opportunities presented by the increasing implication of abnormal phosphorylation in diseases and the development of small molecules to treat these. Abnormal phosphorylation has been implicated in a wide range of diseases and more than 20 hereditary disorders shown to be caused by mutations in kinase and phosphatase genes. Thus specific small molecule intervention in the phosphorylation processes involved in cell signalling offers widespread opportunities for novel therapy in cancer, immunology, inflammation and neurodegeneration.

The day began with an excellent overview on the role that phosphorylation and dephosphorylation play in the body by **Professor Sir Philip Cohen (Dundee, Scotland).** Reversible phosphorylation of proteins catalysed by protein kinases and protein phosphatases regulates almost all aspects of cell life. Scaling up from yeast, which contains 106 kinases and 31 phosphatases, it is estimated that there are 2,000 kinases and between 300 and 500 phosphatases in the human genome. One-third of all proteins can be regulated by reversible covalent phosphorylation, and so each kinase must phosphorylate 15–20 proteins, and the average phosphatase dephosphorylates ~50 proteins. Specific inhibitors of protein and lipid kinases and phosphatases are required to dissect the pathways involved in signal transduction, and this is a major challenge. Examples do, however, exist, such as the immunosuppresant, cyclosporin A binds to cyclophilin and is a specific inhibitor of the phosphatase calcineurin (calcium-dependent protein phosphatase 2B) in T-cells, preventing IL-2 production and blocking T-cell proliferation. This evidence encouraged the further search for specific inhibitors of kinases and phosphatases.

One of the most studied kinase families is that containing the membrane-bound tyrosine kinases (TKs) VEGF, EGF, PDGF and NGF, which are overexpressed in certain cancers. SU101 (1; Leflunomide) currently in Phase-III studies is one such inhibitor. The compound is known for its immunosuppressant effects in rheumatoid arthritis, but it is also very potent in suppression of PDGF-expressing tumours. Specific inhibitors of VEGF and FGF have found a use in angiogenesis and thus may be of benefit in a wide range of diseases, especially where tumour cell growth is dependent upon new blood-vessel generation. Several compounds are in development, such as SU5416, PD173074 and SU5402 (2).

Overproduction of Ras occurs in over 30% of cancers and this pathway, which leads on to the MAP (mitogen-activated protein) kinase system discussed below, is of central

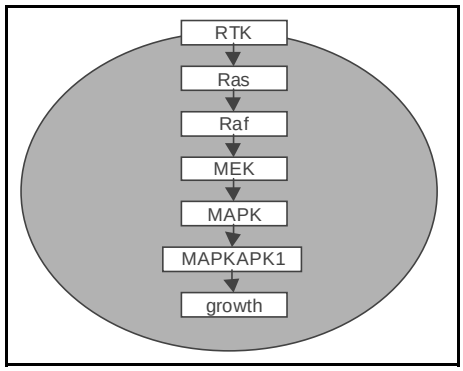

Figure 1 MAP kinase pathway

importance to cell growth and differentiation (Figure 1). Compounds such as PD98059 (3) and U0126 work downstream from the membrane-tyrosine kinases by binding to MAP kinase kinase-1 (MEK) to prevent phosphorylation of the serine residue by Raf. Here these compounds stop one kinase activating another. Targets further downstream interfere with the cell cycle pathway itself, and insofar as such compounds block cellular proliferation, they may have an application in cancer. Purvalanol B (an analogue of olomucine) does not

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distinguish between the cyclin-dependent kinases but is very selective over other (non-cyclin-dependent) kinases.

In hypertension it has been shown that Ca^{2+} sensitisation of smooth muscle is mediated by a Rho-associated protein kinase (RhoK1/p160Rhok). A Yoshitomi compound Y27632 (4) is a potent inhibitor of this kinase and is very selective *in vitro*. In inflammation, the pyridinyl imidazole SB-203580 (5) blocks the production of TNF via inhibiting the stress-activated protein kinase 2 (SAPK2, or p38) and is effective in chronic inflammatory disease models. Despite a close similarity between SAPK3 (p38γ) and SAPK2 (p38α/β), SB-203580 only inhibits SAPK2.

Since the majority of compounds are competitive with ATP, their inhibitory potency is very dependent on the ATP concentration: it is crucial to both measure and quote this factor alongside IC_{50} values. As the ATP binding sites of kinases are highly conserved, the observation of selectivity among some of the above compounds is helpful in understanding the issues associated with mode of action. Analysis of co-crystal structures helps to explain this, contacts outside the ATP binding pocket determine the specificity. A study of the interactions between SB-203580 and amino-acid residues on the kinase have revealed that the fluoryl-benzyl group interacts with a residue outside of the ATP binding pocket (even though the drug is competitive for ATP). Site-directed mutation studies of SAPK2, 3 and 4 have shown the critical nature of Thr106. Most kinases have methionine/ glutamine in this position which prevents access by SB-203580 to the ATP pocket. There are 12 other kinases with Thr106 (e.g., TGFb, Lck, Raf), and all are sensitive to SB-203580. Raf operates via a novel feedback loop to reactivate itself, making it a poor drug target as reported activators may actually be inhibitors.

To evaluate a compound's specificity would require a very large panel of kinases for screening; compounds such as SB-205380 with a known structural requirement can make use of sequence data rather than assays. It is particularly important that compounds used to determine site of action should have known selectivity. For example, although Ro-318220 (6) is used widely as a 'specific' PKC inhibitor, it actually also inhibits kinases from a range of families, such as MAPKAP kinase-1 and p70 S6 kinase. Novel approaches are being used to elucidate *in-vivo* selectivity. One example is the use of stable cell lines producing drug-insensitive forms. The growing list of compounds showing specificity for individual kinases will allow clearer dissection of signalling pathways.

Phosphatases

The second talk, given by **Dr Nick Tonks (Cold Spring Harbor, USA and CEPTYR, USA),** was an exciting update on the potential for protein tyrosine phosphatases (PTPs) as targets for drug discovery. PTPs have been shown to act both positively and negatively in regulation of cellular signalling. They are members of a broad family of enzymes rivalling protein tyrosine kinases in structural diversity and complexity. Genome-sequencing efforts suggest that there will be ~300 PTPs in total, of which ~100 have already been identified. Differences in the regulatory targeting sequences distinguish between different PTPs, of which there are three primary types.

Major progress has been made in determining the crystal structures of PTPs, resulting in detailed mechanistic information. The phosphate group on a tyrosine residue of the target substrate interacts with the signature motif in the active site of the PTP. Substrate binding induces a large conformational change. Cys216Ala and Asp181Ala mutations have decreased activity 1,000fold despite retaining the ability to bind substrate. Experiments with TCPTP and PTP1B have shown that these phosphatases recognise discrete substrates. The structure of a PTP1B-peptide substrate complex also allowed the development of an assay which has revealed that these enzymes display high substrate specificity *in vivo.* This suggests that inhibition of a defined PTP by a drug will only interfere with selected signalling events in cells.

PTEN is a PTP which has been identified as the tumour suppressor on 10q23 and is deleted or mutated in a large number of glioblastoma, endometrial and prostate cancers. PTEN contains the signature motif and is a dual-specificity phosphatase which prefers highly acidic substrates. It has recently been shown that PTEN selectively dephosphorylates the three position of PIP_3. Phosphatidyl inositol phosphates are physiological substrates for PTPs. PTPs can act both positively and negatively and have diverse roles in a wide range of physiological diseases. At CEPTYR they have isolated a number of small molecule leads representing structurally distinct pharmacophores with application to diabetes, obesity, immunosuppression and cancer.

The last session of the morning focused on the physiological role of tyrosine phosphorylation in vascular smooth muscle function. **Dr Alun Hughes (Imperial College, UK)** first gave an overview of smooth muscle cell functions. Vascular smooth muscle cells (VSMC) serve two roles in blood vessels and have distinct phenotypes; differentiated VSMC (contractile) are specialised for sustained force production, to regulate vascular tone and have prominent microfilaments and contractile machinery. Dedifferentiated VSMC (synthetic) participate in the response to injury, are involved in athroma formation, restenosis following surgery and have well-defined Golgi.

Tyrosine phosphorylation is a key intracellular event in the proliferation and migration of VSMC in response to injury. This response is very well characterised in VSMC: medial smooth muscle cell proliferation is the first wave, smooth muscle cell migration is the second wave and intimal smooth muscle cell proliferation is the third wave. There is good evidence that the first-wave mediators are bFGF-receptor TK together with thrombin and angiotensin activating a wide range of TK subsequent to receptor activation. The second-wave mediators are PDGF via receptor TK, together with osteopontin and integrin which activate other TKs, such as src. The third wave is again mediated by PDGF-receptor TK, angiotensin activating src, fyn and jak and TGF-b receptor TK. Response to injury can be shown to be attenuated by a tyrosine

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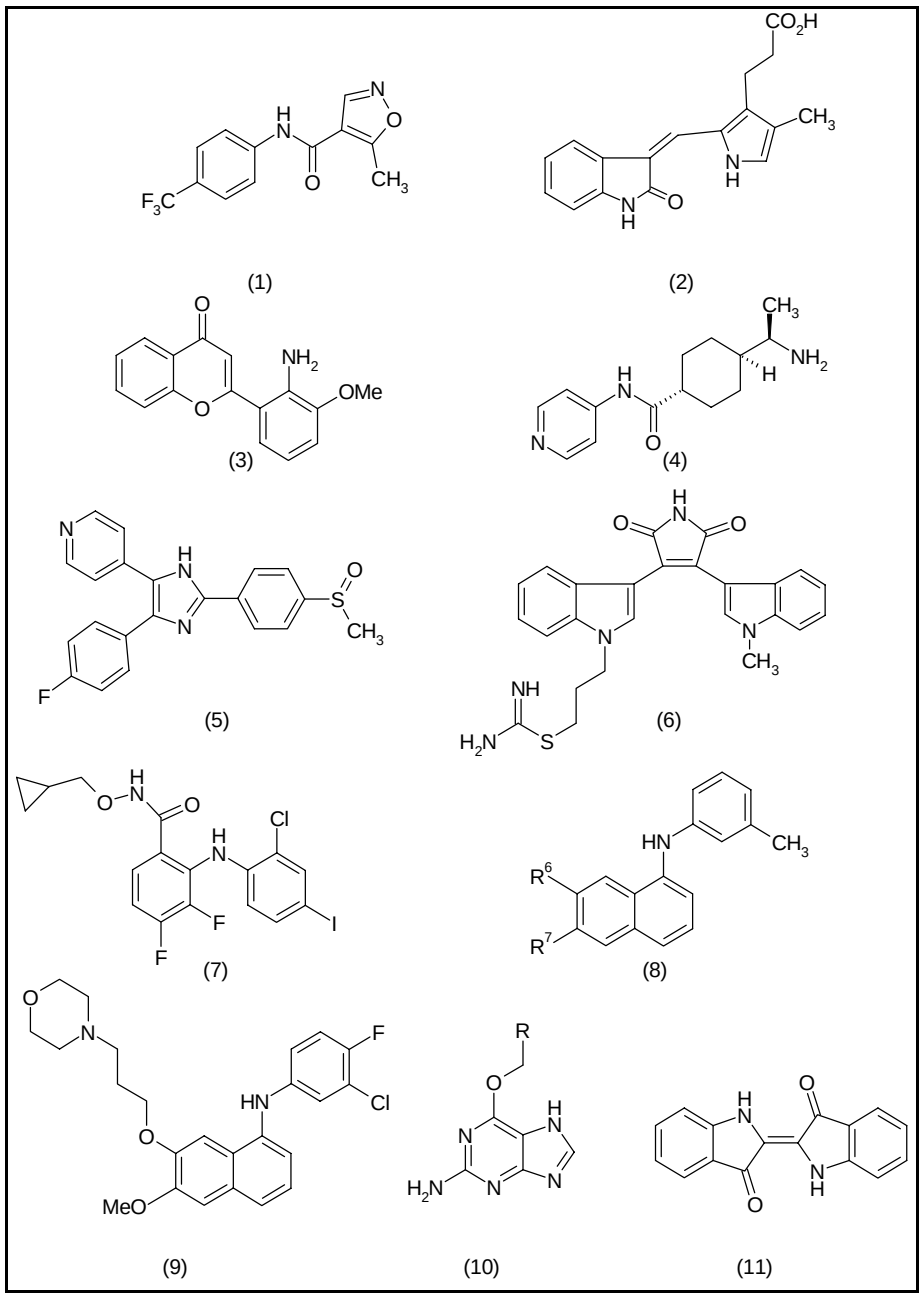

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kinase inhibitor AG-17 which reduced neointima formation following injury. New selective TK inhibitors may open this area to determine the most appropriate points of intervention.

The contraction of smooth muscle cells may also be determined by the degree and/or site of tyrosine phosphorylation within the cell. Growth factors such as PDGF induce a rise in intracellular Ca^{2+} ($[Ca^{2+}]_i$) and contraction in isolated blood vessels as a result of activation of voltage-operated calcium channels. Dr Hughes presented evidence correlating the activity of a range of TK inhibitors with the opening of voltage-operated calcium channels. In most cases the inhibitory potency of s-src corresponded with blockade of calcium channel opening. The non-receptor TK pp60c-src is present in high amounts in smooth muscle and is a likely candidate for the endogenous TK. In addition, contractile agonists (e.g., 5-HT, noradrenaline and angiotensin) also induce tyrosine phosphorylation and the functional responses can be inhibited by the TK inhibitor tyrophostin. Growth factors induce the contraction of isolated blood vessels via activation of calcium channels. There is evidence that src kinase and MAP kinases play an important role in the regulation of intracellular Ca^{2+} and contraction in differentiated VMSC, supporting the potential use of novel selective kinase inhibitors in a wide range of vascular diseases.

Kinase inhibitors in cancer therapy

The afternoon session began with two presentations on the use of protein kinase inhibitors in cancer therapy. Currently available cytotoxic treatments for cancer are limited by toxicity and efficacy. Kinase inhibitors offer potentially improved anti-cancer therapeutics, particularly as signalling targets, and have been proven an effective strategy for hormonal tumours, with a range of targets identified. The first speaker **Dr Judith Sebolt-Leopold (Parke Davis, USA)** gave evidence for specific inhibitors of the MAP kinase pathway-blocking growth of murine and human tumours. MAP kinase kinase (MEK) is a dual-specificity kinase, activated by phosphorylation on Ser218/Ser222. A new class of MEK inhibitors, (4-iodo phenylamino) benzoic acids and their corresponding hydroxamic derivatives have high potency and selectivity for MEK-1. Inhibition of MEK activity has been shown to induce G1 block and thus impair cell-cycle progression. PD184352 (7) is a potent inhibitor of MEK *in vitro* (IC_{50} 0.6nM) with high selectivity compared to a wide range of other kinases. It is not an ATP-competitive inhibitor, nor competitive for the MAPK site on MEK, similar to PD98059 (3). However when MEK-1 mutants were made with glutamate or aspartate substituting for the two serines, PD184352 inhibited their activity while PD98059 did not.

When a panel of murine and human carcinoma cell lines were studied, diverse responses were seen with PD184352. From experiments using PD184352 as a tool to probe basal MAPK levels, it was suggested that cells which do not have high levels of active MAPK do not rely on this pathway for proliferation. High levels of MAPK activity, however, may predict susceptibility of tumour cell lines to PD184352.

Colon scC26 cells are very sensitive to PD184352 *in vivo* (IC_{50} *ca*300nM), while PC3 (prostate) cells are not sensitive to PD184352, suggesting that proliferation in this cell line is not dependent on MAPK. Oral dosing studies (48–400 mg b.i.d.) in the C26 tumour model showed total suppression of MEK up to 9–12 hours post-dose with no toxicity after 14 days'

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dosing. Total inhibition of tumour growth was seen from ~120 mg and the animals were not immunosuppressed. Efficacy correlated with blockade of MAPK activity in excised tumours. This series of compounds appears promising as non-cytotoxic anti-cancer agents in selected tumours. However, the selectivity for MEK-1 over other MEKs was raised during questions from the floor. The suggestion that the resistance to these compounds in some cells could be due to the mix of MEK-1 and MEK-2 rather than just MEK-1 is currently being investigated.

Dr Andy Barker (Zeneca) talked about the epidermal growth-factor receptor (EGFR), which is highly expressed in a range of cancers, particularly non-small-cell lung, colo-rectal, gastric, pancreatic and ovarian types. Aberrant expression of receptor kinase subtypes is common in a range of solid tumours of epithelial origin. Zeneca's programme started from a high throughput screen based on a radio-labelled *in-vitro* assay, from which a lead quinazoline structure was identified. Initial changes in the 6 and 7 positions led to (8; R^6= MeO, R^7=MeO) with kinase IC_{50} of 5 nM, but problems of hydroxylation at the benzylic methyl group compromised *in-vivo* activity. Changing the aniline group to that shown in (9), while keeping the same R^6 and R^7 groups eliminated this problem, and preserved *in-vitro* activity, but poor solubility and logP again reduced activity in the A431 xenograft model. Replacing the 6-MeO group with the a water-soluble morpholine side chain resulted in ZD1839 (9), with kinase IC_{50} of 23 nM and reasonable *in-vivo* activity in the xenograft model. This compound is now in full development, and clinical data due to be released later in the year.

A key aspect of the biology of kinases in inflammation was then dealt with by **Dr Catherine Regnier (INSERM, France)**, whose presentation referred to the research conducted at Tularik in California. This work concerned the activation of the NF-κB protein by I-κB. This signalling pathway has been under intensive investigation because of its central importance in the cellular activation caused by IL-1 and TNF. NF-κB is usually inactivated in the cytosol by binding to I-κB, but upon release from this protein, translocates to the nucleus and activates cytokine transcription. Breakdown of the NF-κB/I-κB complex is facilitated by phosphorylation of I-κB, a process that involves a specific protein kinase. Dr Regnier's talk focused on the identification of this kinase using molecular biological techniques and *in-vitro* experiments. Initial attention centred around a so-called conserved helix-loop-helix ubiquitous kinase (CHUK), which when over-expressed in HeLa cells induced NF-κB activation. CHUK was known to

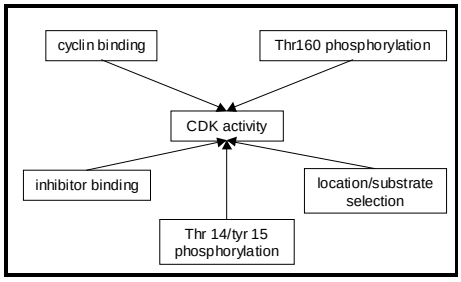

Figure 2 Modulation of Cyclin-dependent kinase activity

mediate in the NF-κB pathway downstream of two other proteins known as NIK (NF-κB- inducing kinase) and TRAF. A series of elegant experiments showed that CHUK interacts with — and phosphorylates at — the serine residues 32 and 36 the α form of I-κB, and was renamed IKKα. Subsequently, IKKα has been shown also to phosphorylate the ß form of I-κB known as I-κBß, and another kinase has been identified called IKKß, which is selective in the phosphorylation of I-κBß. IKKα and IKKß can form homo- and heterodimers, while the latter are preferred. NIK stimulates phosphorylation of both IKKα and IKKß, the former predominating. *In vivo,* a complex of IKKα and IKKß, together with NIK, NF-κB and relA is likely to exist. High-throughput screening is under way to identify inhibitors of IKKα and IKKß, and IKK-knock-out animals have been produced in collaboration with Roche Bioscience. During the discussion that followed this talk the limitations of the purely *in-vitro* methods that led to these discoveries were highlighted, and it was acknowledged that confirmation of this model would require the discovery of selective inhibitors of these various kinases, so that their importance in a whole organism could be determined. Work on such compounds is under way at Tularik, and leads have been identified.

The final talk of the day explored the design of novel inhibitors of kinases through molecular modelling. **Dr Martin Noble (University of Oxford)** concentrated upon the modelling of cyclin-dependent kinases involved in cellular proliferation. Cyclin-dependent kinase activity is modulated by a number of mechanisms (Figure 2), all of which are reversible. For example, the activity of the transcription factor E2F-1 is modulated through binding to DP-1 which is phosphorylated by CDK-4 and CDK-6, and the protein RB which also binds to the complex.

CDK-2 is a well-characterised kinase which is inhibited by stautosporine. X-ray crystal-structure analysis shows that the binding of stautosporine extends well into the kinase active site, and suggests that after binding the conformation of the protein changes radically from a flat to a heavily folded shape. One point to consider regarding specificity was that the lysine-89 residue that lies near the active ATP-binding domain varies quite considerably among kinases. Dr Noble's talk examined in some detail the binding of purine-based CDK inhibitors such as (10), in which the R group included cycloalkyl and branched alkyl chains. The hydrophobic interactions of such groups with the site otherwise occupied by the ribose group of ATP suggests that direct chemical similarity with the sugar moiety is not essential for activity.

Another interesting insight into the mechanism of action of these enzymes came from studying a Chinese herbal remedy for leukaemia. Qing Dai derives much of its activity from indirubin (11) — a structural analogue of indigo. Indirubin is an inhibitor of CDK-2, for which cyclin A is a substrate, with an IC_{50} of 2.2 μM.

In conclusion, this was a successful meeting that drew a deservedly large audience of about 150 to see an internationally renowned group of speakers present contemporary findings in an intensively investigated area. Although still at an early stage, and clearly enormously complex, there is great scope for innovation and the promise of real drug discovery from this area.•

Angiogenesis cont'd

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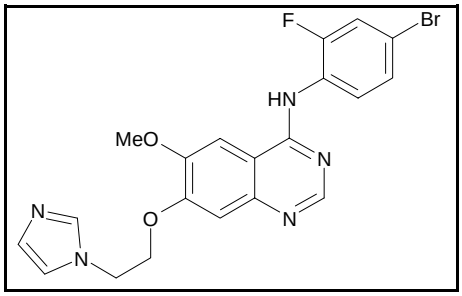
the *corpus rubrum* and *corpus luteum* but Ang2 is upregulated during vessel regression.

Tumours with greatest vasculature are generally associated with poorer prognosis and increased metastasis and a prognostic marker is needed. Several techniques, including dual labelling of proliferating endothelial cells have been developed to identify specific markers for angiogenesis.

A possible marker is monocyte chemo-attractant protein-1 (MCP-1). It is a more potent inducer of angiogenesis than either VEGF or bFGF. In corneal angiogenesis, induction of angiogenesis by MCP-1 is concomitant with a massive inflammatory response and macrophage recruitment. With VEGF an increase in angiogenesis occurred but there was an absence of macrophage recruitment. There are distinct qualitative differences in the contribution of an inflammatory response to the induction of angiogenesis, which differentiates physiological and pathological angiogenesis.

Rheumatoid arthritis affects ~2% of Western populations, and osteoarthritis of one or more joints is almost inevitable by the age of 70. Current treatments for these conditions are palliative and not cures. Angiogenesis is important in the underlying causes of arthritis (inflammation, joint damage). These underlying causes, reasons for its persistence, severity of inflammation and joint damage were all topics covered by the



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next speaker **Dr David Walsh (City Hospital, Nottingham)**. Neovascularisation of inflammatory synovium is a hallmark of the disease. While cartilage is avascular, the synovium has one of the densest vasculatures in the body. Many stimulators of angiogenesis are expressed in synovial tissue — e.g. VEGF and integrin $\alpha_v\beta_3$ — but it is not clear which are the most important inhibitors.

Rheumatoid synovium has focal hypoxia and a defective vasoregulatory system, specifically, immature vessels are not able to be regulated efficiently and consequently do not oxygenate/remove metabolites effectively. There is also a focal absence of nerves. New vessels are laid down inappropriately, and the pannus begins to invade the cartilage and bone. Innervation occurs very slowly. In turn, hypertrophic chondrocytes and osteoblasts generate angiogenic factors which stimulate ossification.

It may be possible to knock-out early stage synovial angiogenesis, thus preventing persistence. Integrin $\alpha_v\beta_3$ antagonists have been shown to inhibit antigen/bFGF arthritis (Storgard et al. (1999) *J. Clin. Invest.* 103: 47–54). This and other studies confirm that pharmacological angiogenesis inhibition is a viable therapeutic strategy for rheumatoid arthritis.

Dr Margaret Rees (John Radcliffe Hospital, Oxford) described the key factors associated endometrial angiogenesis. One in five menopausal women, by the age of 55, undergoes hysterectomy for excess bleeding, which results in 50,000 hysterectomies being performed each year. Menstrual bleeding is a phenomenon restricted to humans and sub-human primates which have coiled blood vessels or spiral arterioles in their endometria. These undergo growth or angiogenesis throughout the menstrual cycle and are under the control of endogenous and exogenous steroids — oestradiol and progesterone.

Many angiogenic factors are present in the endometrium. The three major players in endometrial angiogenesis are VEGF, thymidine phosphorylase and adrenomedullin.

A number of endometrium models have been developed by culturing epithelium from this tissue (hysterectomy specimens). It was found that the cells were uniquely responsive to VEGF and that VEGF-A was upregulated by steroids, for example, oestradiol. Strong induction of the angiogenic enzyme thymidine phosphorylase was observed using a combination of IFNγ and TNFα. There was no effect on expression using oestrogen or progesterone but the latter in combination with TGFβ caused an increase in enzyme levels. Adrenomodullin is a novel growth factor for endothelial cells which shows potent angiogenic activity *in vivo* in the chick chorioallantoic membrane assay.

The role of CXC chemokines in the regulation of angiogenesis was discussed by **Dr Douglas Arenberg (University of Michigan, USA)**. Net neovascularisation is determined by the balance of angiogenic and angiostatic stimuli. CXC chemokines containing an ELR motif (ELR+) immediately preceding the CXC motif, for example, IL-8, Gro α/β/γ, ENA-78, are capable of stimulating angiogenesis. Those which do not contain the ELR motif (ELR-), for example, IP10, MIG and PF4, are angiostatic. PF4 inhibits angiogenesis induced by ELR+ chemokines. Tumour progression is associated with over-expression of ELR-CXC and under-expression of non-ELR-CXC; in tumour regression this scenario is reversed.

In non-small-cell lung cancer, IL-8 and ENA-78 levels were increased compared to normal. Using SCID mice injected with A549 adenocarcinoma cells, the rate of tumour growth was directly proportional to IL-8 levels. Anti-IL-8 antibody decreased tumour growth.

In a model of idiopathic pulmonary fibrosis (IPF) the lungs were 'primed' for angiogenesis. Removal of IL-8 decreased angiogenesis. In contrast, a decrease in IP-10 increased angiogenesis. Mutagenesis studies revealed that the arginine of the ELR motif in CXC was most important for receptor binding and that the whole chemokine molecule is required for activity. The ELR peptide is not sufficient on its own to have an effect (compare RGD mimetics).

Dr David Ogilvie (Cancer and Infection Research, AstraZeneca) focused on the inhibition of VEGF signal transduction in solid tumour disease, particularly in non-hormone-dependent cancers. There is strong evidence that VEGF contributes to tumour growth through the promotion of both angiogenesis and vascular permeability (*Cancer and Metastases Review* (1993) 12: 303). Its sequestration has been

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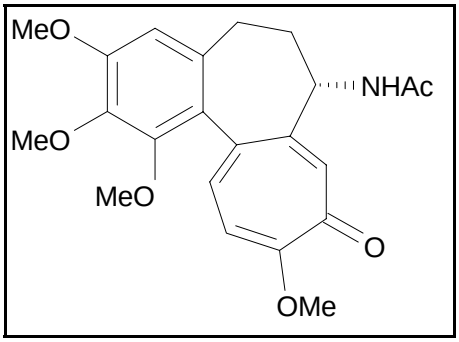
shown to reduce tumour growth in animal models (Kim et al. (1993) *Nature* 362: 841). A VEGF antibody is in Phase-II clinical trials. AstraZeneca scientists have designed a number of inhibitors of VEGF receptor associated tyrosine kinase (RTK) Flt. The compounds are competitive with respect to



Colchicine

ATP. These compounds, which arose from a EGFR programme, have a different profile of inhibition on the two receptors showing selectivity is possible. Moreover these differences translated into different *in-vivo* profiles in xenograft tumour models, and at doses unlikely to affect tumour cells directly.

In summary, ZD4190, an orally active inhibitor of VEGF signal transduction, displays a non-cytotoxic, pan-carcinoma anti-tumour profile, and activity consistent with the proposed mode of action.

Dr David Chaplin (Rhône-Poulenc Rorer, France) discussed the potential for agents that can target and alter the function of already formed vasculature. A key advantage to this approach is that damage to a single vessel can kill thousands of tumour cells.

There have been a number of antibody-based and gene-therapy approaches to vascular targeting. Drug-based approaches have included flavanoids, xanthenones, serotonin agonists and tubulin binding agents — for example, colchicine — which cause haemorrhagic necrosis in tumours. These compounds work in all tumours but only at their maximum tolerated dose. A novel class of tubulin inhibitors has been identified, called combretastatins, which come from the bark of the African Bush Willow.

One of the most effective agents is combretastatin CA4P, a soluble form of combretastatin CA4, which inhibits tumour growth at 0.10 of its maximum tolerated dose. It was found to be most effective on immature vessels. CA4P induces an increase in blood-flow resistance going into the tumour, but not into the rest of the vasculature. The effect was immediate and observed within 20 minutes. These studies confirm the potential of CA4P as a neovasculature targeting agent and have highlighted that endothelial cell shape changes are a key element in its mode of action.

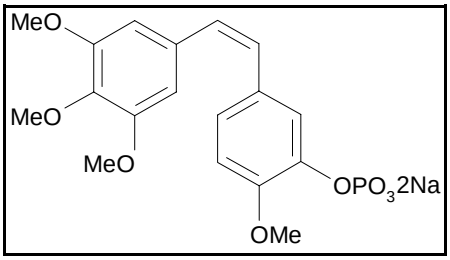
Dr Wen Jiang (University of Wales College of Medicine, Cardiff) discussed the regulation of tumour- and angiogenic factor-induced angiogenesis and its implication in wound healing. A factor involved is hepatocyte growth factor/scatter factor (HGF/SF) produced by stromal fibroblasts. The protein has four-unit kringle domains (K1-K4) with a N-terminal hairpin (N). It is produced as a pre-protein and processed into a α and β subunits, held together by a disulphide bridge. It is mediated by the C-Met receptor protein. The α subunit (N-K1) is responsible for receptor binding. HGF/SF is found to increase motility, integrin expression and invasion in tumour cells. In endothelial cells, HGF/SF increased motility and CD44 expression and decreased the gap junctions.

HGF/SF was produced in a soluble form, NK4, and used as an antagonist of HGF/SF in an *in-vitro* tube-forming assay. NK4 reduced cell motility and hence tube length. Fibroblasts, which are good suppliers of HGF/SF and matrix embedded fibroblasts, enhance the tubule formation of endothelial cells and new vessel formation from tissues of acute and chronic wounds. It is possible that matrix-bound factors — for example, HGF/SF — are released upon stimulation in a wound environment indicating a possible role in the intervention of wound healing.

Professor Eva Kohner (St Thomas's Hospital, London) discussed the pathogenesis, treatment and possible prevention of new vessel formation in the diabetic retina. The two most critical factors in diabetic retinopathy are maculopathy (leakage) and new vessel formation, which are the result of ischaemia/hypoxia, and indirectly, high glucose. Abnormalities in blood components are seen, such as reduced red-cell deformability, increased platelet aggregation, decreased white-cell deformability and increased white-cell adhesion.

Recent studies suggest that up-regulation of cor-2 enzyme by high glucose in the larger white cells, alters glycoproteins in the cell membrane causing increased adherence to the vessel wall *via* I-CAM and selectin. This results in capillary occlusion. Hyperglycaemia also causes dilation of the capillaries, resulting in increased blood flow that causes sheer-stress and damage to the endothelium and to the pericytes, which control endothelial function and proliferation. An increase in the number of pericytes is the earliest abnormality observed in diabetes. They are damaged primarily by non-enzymatic glycation and extra- and intracellular advanced glycation end-products (AGE) formation. Ischaemia results in the liberation of a host of vasoproliferative factors, the most important of which is VEGF. Protein kinase-C (PKC) β_2 enzyme is necessary for increased VEGF expression and is activated by hypoxia and ischaemia. New vessels tend to grow into the posterior of the vitreous which contracts, pulling on the retinal vessels resulting in haemorrhage and retinal detachment, ultimately leading to visual loss.

There are only two treatments for diabetic retinopathy. One is laser photocoagulation which destroys the ischaemic retina and allows the accumulation of angiostatic substances. New blood vessels close, shrink and disappear. The other is to control glucose



C4AP

levels, and in the process, to minimise glucose toxicity control high blood pressure.

A novel PKC β_2 inhibitor is under clinical trial at present. The role of growth hormone (GH) in ischaemia-associated retinal vascularisation has been studied suggesting that GH receptor antagonists may also have therapeutic potential.•

(continued from page 1)
1,000 pupils and teachers were enthralled by a series of vivid demonstrations of energy changes, which produced luminescence, heat and light.

A number of scientific suitcases, containing a collection of anatomical models used in teaching students about the human body has been purchased. These are freely available to local schools to be borrowed and have proved very popular with teachers who maintain that they add interest to their lessons. As an example of the primary committee's work, SB at Harlow co-hosts, along with other companies under the auspices of Science and Technology Regional Organisations (SATRO), a number of workshops contributing to 'The Delights of Science', an annual event open to all Year 6 and Year 7 pupils in Harlow.

To stimulate a continued interest in science the secondary committee is involved in a number of events during the school year. SB is a major sponsor of a two-day Creative Science programme held at Harlow College where Year 8 students from schools in the surrounding areas experience a structured set of investigations encompassing all scientific disciplines.

These experiments are designed to extend the students' knowledge and to introduce them to the benefits of teamwork. On the satisfactory completion of an investigation report based on their findings, the students are awarded a bronze Creativity in Science and Technology (CREST) certificate, a established national award scheme supported by the government through SATRO.

Building on the success of the 'Delights of Science', the secondary committee hosts an annual series of lectures entitled 'Excite your Senses' where over 1,300 Year 9 students take part in two Royal Institution-style lectures designed to stimulate scientific interest within this key age group. For older pupils, SB participates in a work-experience programme in which schoolchildren in Years 10 and 11 (15/16-year-olds) work alongside SB scientists for a week. The week is structured around the work-experience learning framework run by the Department of Education and Employment, which SB supports, together with the Centre for Education and Industry at the University of Warwick. The students find this week invaluable and get real insight into how the pharmaceutical industry works.

In addition, young scientists' days are organised to allow sixth-form students to participate actively in a small research-based project. This allows them to experience research work within a large pharmaceutical company and take part in a business game, which shows the decision processes involved in drug discovery.

In 1999, SB is sponsoring 'Visions for the Future of Health', a conference organised by the British Association at which students from a number of European countries visit Harlow and find out about our current understanding of genetics.

Involvement in school liaison is an established part of many pharmaceutical employees' lives, allowing them to encourage and influence the scientific development of young people within their own workplace. Contact schools value the help and support provided by SB and their feedback on current events has been invaluable. Initiatives such as those outlined above stimulate and maintain the interest of schoolchildren in science, which can only benefit the pharmaceutical industry in the long term.

SmithKline Beecham Pharmaceuticals, Harlow, Essex CM19 5AD•

Endnote: Newsletter available electronically

Advances in technology have allowed the printing of the SMR Newsletter in pdf format. Cognescenti of the Internet will be aware of this versatile and compact format for documents. It can be used with Adobe Acrobat reader, which if not present already on your system can be downloaded free of charge from the Internet. We shall be posting the newsletter on the SMR home page. If SMR members would like to receive future issues in electronic form please send an e-mail to Chris Ryan at c.ryan@iob.org. While on the subject, please also note that it is also now possible to register electronically for SMR meetings and send membership application forms *via* the SMR home page. We hope you like these developments.•

Notes

DIARY

5–9 September 1999. Receptor Chemistry towards the Third Millennium, Camarino, Italy. Contact: 12th Camerino-Noordwijkerhout Symposium, Dipartimento di Scienze Chimiche, Universita di Camerino, Via S. Agostino, 1-62032 Camerino, Italy.

15 September 1999. Molecular Strategies for Drug Discovery and Design, Hatfield. Contact: Dr R. Rapley, Department of Biosciences, University of Hatfield, College Lane, Hatfield AL10 9AB.

10–14 October 1999. Sixth International Conference on Endothelin, Montreal, Canada. Contact: Dr B. Battistini, Secretary-General, Hôpital Laval, Institut de Cardiologie et de Pneumologie, Centre de Recherche Clinique et Fondamental, 2725 Chemin Ste-Foy (Québec), Canada G1V 4G5.

NEW MEMBERS

British Biotech: A. Wright. *Celltech Therapeutics Ltd:* A. Foley, M. Hutchings. *CRC Centre for Cancer Therapeutics at ICR:* M. Garrett. *Ilex Oncology:* C.J. Wareing. *Imperial College London:* A.D. Hughes. *H. Lundbeck A/S:* E.B. Nielsen. *Marie Curie Research Institute:* N.D. Brewis, A. Phelan. *Organon Laboratories:* F.H. Sansbury. *OSI Pharmaceuticals:* G.M. Wynne. *Pfizer:* J.P. Overington, P. Vickers. *The Rayne Institute:* K. Jahangir. *SmithKline Beecham:* J.R.S. Arch, C.A. Armour, S. Ashman, C. Briscoe, S.J. Charlton, D. Cross, S. Dowd, D. Haigh, J. Holder, J.P. Hughes, A. Kalender, G.F. Joiner, G.J. Murphy, A. Naylor, K. Philpott, M. Ray, A. Reith, O. Rausch, A. Rowles, C.A. Smith, C. Southan, P.C. Staton, M. Tadayyon, R.V. Ward, M.G. Wilkinson. *St Thomas's Hospital:* A. Smith, M. Waltham. *University College London:* K.N. Bussell, M.D. Lobo. *University of Greenwich:* M.J. Davies. *University of Oxford:* E. Vernon-Wilson. *Vertex Pharmaceuticals (Europe) Ltd:* T. Hercend, K. Miller. *Williams De Broë:* S.P. Conway. *Yamanouchi Research Institute:* S. Beach, I. Bird, G. Craggs, S. Gadher, S. Kellie, G. O'Sullivan, H. Scotney. *No affiliation supplied:* K. Hellmann.

NEWSLETTER PRODUCTION

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