BMS 1 Project Title: PURIFICATION AND IDENTIFICATION OF NATURAL PRODUCTS FROM HONEY AND PROPOLIS WITH ACTIVITY AGAINST GRAM-POSITIVE BACTERIA

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Project Area: (one or more of the following)
Biomedical Analysis /Microbiology

Project Description:
There is now an urgent need to discover and develop some novel antimicrobial agents to tackle community and healthcare-associated infections caused by Gram-positive bacteria (e.g. MRSA, VRE). Investigating natural sources is an approach which offers a unique source of chemically diverse molecules that could become new drug leads. Products from the beehive, such as honey and propolis, have been used for centuries in traditional medicine worldwide for their healing properties. Their antimicrobial activity, against MRSA strains for example, has become the subject of recent scientific interest[1,2]. However, the exact nature of the antimicrobial chemical constituent(s) has never been fully characterised. The purpose of this project is to;

i) fractionate some honey and propolis samples from various origins using a range of purification procedures
ii) elucidate the structures of the natural products isolated using a combination of spectroscopic techniques
iii) determine the Minimum Inhibitory Concentrations (MICs) for all samples against a range of Gram-positive bacteria and interpret the results according to the recommendations of the BSAC guidelines[3].

Techniques to be used:
Analytical techniques; High-pressure liquid chromatography(HPLC), liquid-liquid partition, vacuum liquid chromatography (VLC), gel filtration, open column chromatography (CC), ion-exchange chromatography, thin-layer chromatography (TLC), Infra-red and ultra-violet spectroscopy (IR, UV), mass spectrometry (MS) and high-field nuclear magnetic resonance (NMR) spectroscopy.
Microbiological techniques; *In vitro* antibacterial screening assay using the agar dilution method against bacteria including susceptible *Staphylococcus aureus* NCTC 6571 and EMRSA-16.

References:
1. Seidel V, Peyfoon E, Watson DG, Fearnley J. Comparative study of the antibacterial activity of propolis from different geographical and climatic zones. Phytother Res. 2008 Jun 20. [Epub ahead of print]

2.Maeda Y, Loughrey A, Earle JA, Millar BC, Rao JR, Kearns A, McConville O, Goldsmith CE, Rooney PJ, Dooley JS, Lowery CJ, Snelling WJ, McMahon A, McDowell D, Moore JE. Antibacterial activity of honey against community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA). Complement Ther Clin Pract. 2008 14:77-82.

3. Andrews JM. BSAC standardized disc susceptibility testing method (version 4). J Antimicrob Chemother 2005; 56: 60–76.

BMS 2 **Project Title:** Characterisation of a novel mechanism for modulating the activity of pain-sensing nerves

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Project Area: (one or more of the following)
Pharmacology

Project Description:
Overview: At present the therapeutic options for treating chronic pain are limited, but new targets have recently been identified, including P2Y receptors and 2-pore potassium ion (K_{2P}) channels. Both are expressed in the pain-sensing C and Aδ sensory nerve fibres, which have their cell bodies in the dorsal root ganglia (DRG) and so are potentially important targets for controlling the activity of pain-sensing nerves. Our MRes projects in previous years in this area were highly successful and produced sufficient novel data for a research paper. Now we aim to continue these studies and further develop our understanding of the interaction between P2Y receptors and K_{2P} channels.
Background: P2Y receptors are a family of G protein-coupled receptors that are activated by nucleotides, such as adenosine 5'-triphosphate (ATP) (Abbrachio *et al.,* 2006). K_{2P} channels are highly selective for K^+ ions and make a substantial contribution to the neuronal resting membrane potential in many regions of the CNS (Kim, 2005). We have shown that native, ATP-sensitive P2Y receptors inhibit the K_{2P} background current normally present in rat cerebellar granule neurones and that recombinant human $P2Y_1$ and $P2Y_{12}$ receptors inhibit currents carried by K_{2P} channels co-expressed in a cell line (Parmar *et al.,* 2008). Both P2Y receptors (Kennedy, 2008) and K_{2P} channels (Alloui et al., 2006) are expressed in sensory neurones.
Aim: The aim of this project is to characterise how P2Y receptors and K_{2P} channels interact to modulate cellular activity. The student will use the patch clamp technique to record ion currents carried by K_{2P} channels and characterise their modulation by P2Y receptor activation. This will be supported by the use of recombinant channels and receptors. They will also learn tissue-culture and will use immunohistochemical techniques to study native protein expression. Together, these experiments will help advance the search for new, effective analgesics.

Techniques to be used:
Patch-clamp electrophysiology, molecular biology (expression of recombinant receptors & ion channels), tissue-culture, immunohistochemistry

References:
1. Abbracchio, MP *et al.,* (2006). Update of the P2Y G protein-coupled nucleotide receptors: from molecular mechanisms and pathophysiology to therapy. *Pharmacol. Rev.,* **58**, 281-341.
2. Kennedy, C. (2008). $P2X_3$ receptors and sensory transduction. In: *Sensing with Ion Channels.* ed. Martinac, B. (Springer, Heidelberg), 247-266.
3. Kim, D. (2005). Physiology & pharmacology of two-pore domain potassium channels. *Curr Pharm Des.* **11,** 2717-36.
4. Alloui A *et al.,* (2006). TREK-1, a K^+ channel involved in polymodal pain perception. *EMBO J.,* **25,** 2368-76.
5. Parmar, M., Kennedy, C. and Bushell, T. (2008). 2-pore potassium ion channels are inhibited by both $G_{q/11}$- and G_i-coupled P2Y receptors. *(submitted for publication).*

BMS 3 Project Title: Examining the effects of IGFBP-5 in epithelial-mesenchymal transition

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Project Area: (one or more of the following)
Biochemistry/Pharmacology

Epithelial cells serve a variety of functions, one of which is to act as a barrier to infection and external insults. Injury, including death of epithelial cells, provokes a wound-healing response which results in migration of cells into the wound site in an attempt to seal the injured tissue. This process also activates the underlying cells (principally fibroblasts) to secrete collagen and fibronectin, proteins which help to plug the wound. If this is not controlled, excessive deposition of these proteins can lead to scarring (fibrosis) and can lead to death in, for example, lung fibrosis or cirrhosis of the liver. It is now apparent that chronic damage to a variety of epithelial cells leads to fibrotic disease but that this is the result of a relatively minor but repetitive injury which does not activate inflammation to any great extent. Instead it appears that a factor(s) produced by injured epithelial cells stimulates the underlying collagen-producing cells, resulting in fibrosis, which progressively impairs the function of major organs such as the lungs, kidneys and liver. This repetitive stimulus to cell proliferation may also be a trigger for premature cell aging (senescence) which has recently been linked to fibrosis. Our focus will centre on a protein, IGFBP-5, secreted by the epithelium, which plays a significant role in cell death and has been proposed to induce fibrosis in the adult and is also implicated in the process of cell aging. This mechanism is consistent with the late-onset of fibrotic diseases, which typically occur from middle-age onwards. We will also examine the effect of epithelial damage on the response of the underlying tissue, which contains the collagen-secreting fibroblasts. Responses will include changes in cell proliferation, migration and aging, as well as expression of various genes and proteins implicated in these events. The use of confocal microscopy will allow us to visualise these changes in living cells in complex reconstructions of the tissues under study. This proposal thus seeks to provide evidence that IGFBP-5 is a pivotal initial response to epithelial injury and that, by understanding this process, we will be able to develop novel approaches to deal with the consequences when this repair mechanism malfunctions in processes such as fibrosis and cell aging.

Techniques to be used: cell culture, immunocytochemistry, PCR, Western blotting

References:

Yasuoka H, Jukic DM, Zhou Z, Choi AM & Feghali-Bostwick CA 2006a Insulin-like growth factor binding protein 5 induces skin fibrosis: A novel murine model for dermal fibrosis. *Arthritis Rheum* **54** 3001-3010.

Kim KS, Seu YB, Baek SH, Kim MJ, Kim KJ, Kim JH & Kim JR 2007 Induction of cellular senescence by insulin-like growth factor binding protein-5 through a p53-dependent mechanism. *Mol Biol Cell* **18** 4543-4552.

BMS 4 Project Title: Determination of the effects of IGFBP-5 on fibroblast activation in fibrotic disease

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Project Area: (one or more of the following)
Biochemistry/Pharmacology

Epithelial cells serve a variety of functions, one of which is to act as a barrier to infection and external insults. Injury, including death of epithelial cells, provokes a wound-healing response which results in migration of cells into the wound site in an attempt to seal the injured tissue. This process also activates the underlying cells (principally fibroblasts) to secrete collagen and fibronectin, proteins which help to plug the wound. If this is not controlled, excessive deposition of these proteins can lead to scarring (fibrosis) and can lead to death in, for example, lung fibrosis or cirrhosis of the liver. It is now apparent that chronic damage to a variety of epithelial cells leads to fibrotic disease but that this is the result of a relatively minor but repetitive injury which does not activate inflammation to any great extent. Instead it appears that a factor(s) produced by injured epithelial cells stimulates the underlying collagen-producing cells, resulting in fibrosis, which progressively impairs the function of major organs such as the lungs, kidneys and liver. This project will examine aspects of the activation of fibroblasts during this process. Our focus will centre on a protein, IGFBP-5, secreted by the epithelium, which plays a significant role in cell death and has been proposed to induce fibrosis in the adult and is also implicated in the process of cell aging. This mechanism is consistent with the late-onset of fibrotic diseases, which typically occur from middle-age onwards. Responses will include changes in cell proliferation, transdifferentiation, migration and aging, as well as expression of various genes and proteins implicated in these events. The use of confocal microscopy will allow us to visualise these changes in living cells in complex reconstructions of the tissues under study. This proposal thus seeks to provide evidence that IGFBP-5 is an important activator of fibroblasts and that, by understanding this process, we will be able to develop novel approaches to deal with the consequences when this repair mechanism malfunctions in processes such as fibrosis and cell aging.

Techniques to be used: cell culture, immunocytochemistry, PCR, Western blotting

References:

Yasuoka H, Jukic DM, Zhou Z, Choi AM & Feghali-Bostwick CA 2006a Insulin-like growth factor binding protein 5 induces skin fibrosis: A novel murine model for dermal fibrosis. *Arthritis Rheum* **54** 3001-3010.

Kim KS, Seu YB, Baek SH, Kim MJ, Kim KJ, Kim JH & Kim JR 2007 Induction of cellular senescence by insulin-like growth factor binding protein-5 through a p53-dependent mechanism. *Mol Biol Cell* **18** 4543-4552.

BMS 5 Project Title: Development and characterisation of fluorescently-tagged VPAC/PAC receptors for *in vivo* and *in vitro* analysis

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Project Area: (one or more of the following)
Biochemistry /Pharmacology/Molecular Biology

Project Description:

Research in our laboratory has focussed on defining the molecular mechanisms that determine the function of two G protein-coupled receptors (GPCRs), the PAC_1 and $VPAC_2$ receptors [1-4]. These receptors mediate the actions for two structurally related neuropeptides, pituitary adenylate cyclase activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP). PAC_1 and $VPAC_2$ receptors share ~50% amino acid sequence similarity, however there are marked functional differences with respect to their distribution, ligand selectivity, levels of expression and G protein-coupling. In particular, the PAC_1 receptor is selective for PACAP and VIP is approximately 1000-fold less potent at this receptor, whereas the $VPAC_2$ receptor is a high affinity receptor for both PACAP and VIP. In addition, several amino terminal domain and intracellular loop 3 splice variants exist for the PAC_1 receptor, increasing receptor diversity [4]. We are investigating the role of different receptor domains in determining their ligand selectivity and G protein-coupling [2,4].

Traditionally, assays used to determine receptor expression, localisation, trafficking and internalisation rely on radioactively-tagged ligands and/or on antibodies that bind specifically and with high affinity to the receptor protein. These assays are useful also for determining the effects of mutations on receptor functions, such as receptor expression/trafficking, ligand binding and activation of intracellular signalling pathways. However, suitable ligands and/or antibodies may not be available or can be expensive to obtain and the assays need to be stopped before measurements are taken. The use of variants of the Green Fluorescent Protein (GFP) that can be fused to the target protein by recombinant techniques and also fluorescent tags that can be covalently linked to amino acid sequences present in peptides, such as the FlAsH method [5] has enabled the development of several types of assays that capitilise on the ability to excite the fluorescent tag at a specific wavelength. Moreover, assays utilising these can be conducted in live cells in real time. Multiple labels that have different excitation/emission spectra can be utilised at the same time because they can be individually detected. In addition, fluorescent methodologies, such as fluorescence resonance energy transfer (FRET) and fluorescent polarisation (FP), have been developed so that more refined biological questions regarding ligand binding and protein:protein interactions can be answered [6].
The aim of this project is to develop and characterise fluorescently-labelled PAC_1 and $VPAC_2$ receptors for use in *in vivo* and *in vitro* functional studies. Fluorescently-tagged receptors will be used to visualise receptor localisation in whole cells using fluorescent microscopy in order to determine cell surface expression and internalisation following receptor activation. The receptors will be tagged by fusion with variants of the fluorescent protein GFP. In addition, the amino acid sequence CCPGCC will be incorporated into the 3rd intracellular loop or at the carboxyl tail terminus in order to attach the FlAsH fluorophore. Biological activity of the recombinant receptors will be determined

in second messenger assays and expression monitored in whole cells and on Western blots.

Techniques to be used:

The project will involve molecular biology techniques (PCR and cDNA manipulation) and biochemical techniques (cell culture and transfection, fluorescent microscopy, second messenger assays, Western blotting).

References:

[1] Lutz, E.M., Sheward, W.J., West, K.M., Morrow, J. A., Fink, G. & Harmar, A.J. (1993) *FEBS Lett.* **334**, 3-8.

[2] Lutz, E.M., MacKenzie, C.J., Johnson, M., West, K., Morrow, J.A., Harmar, A.J. & Mitchell, R. (1999) *Br. J. Pharmacol.* **128**, 934-940.

[3] MacKenzie, C.J., Lutz, E.M., Johnson, M. S., Robertson, D.N., Holland, P.J. & Mitchell, R. (2001) *Endocrinology* **142**, 1209-1217.

[4] Lutz, E M, Ronaldson, E, Shaw, P, Johnson, M S, Holland, P J & Mitchell, R (2006) *Mol. Cell Neurosci*. **31**, 193-209.

[5] Adams, S. R., Capmbell, R. E., Gross, L. A., Martin, B. R., Walkup, G. K., Yao, Y., Llopis, J. and Tsien, R. Y. (2002) *J. Am. Chem. Soc.* **124**, 6063-6076.

[6] Miyawaki, A. (2003) *Dev. Cell* **4**, 295-305.

BMS 6 Project Title: The role of phospholipids in streptomycete growth and differentiation

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Project Area: (one or more of the following)
Biochemistry/~~Biomedical Analysis/Food Science/Immunology~~/Microbiology/Molecular Biology/~~Parasitology/Pharmacology~~

Project Description:

The involvement of lipids in bacterial cell division has recently received increasing attention, for example the components of the *Escherichia coli* cell division machinery are functionally dependent on membrane lipids (Mileykovskaya & Downham, 2005). Although different phospholipids are abundant in the membranes of the Gram positive, differentiating bacterial genus *Streptomyces*, their involvement in growth, differentiation and sporulation is not known. In order to investigate the role of phospholipids in streptomycete cell division we will apply a combination of microscopic, genetic and biochemical techniques in the model organism *Streptomyces coelicolor* (Bentley *et al.*, 2002).

Localization of phospholipid domains in streptomycete hyphae. *Streptomyces coelicolor* substrate and aerial hyphae will be stained with phospholipid specific dyes in conjunction with other stains that localize to cell division components. Fluorescent microscopy of stained substrate and aerial hyphae will then be used to localize phospholipids in relation to other cell division markers. Phospholipid incorporation into cell membranes will be studied by time lapse microscopy.

Localization and function of phospholipid biosynthetic enzymes. Mutations in genes putatively involved with phospholipid biosynthesis will be created by Tn*5062 in vitro* transposition and transferred into *S. coelicolor* by conjugation and allelic replacement (Bishop *et al*., 2004). Translational fusions to the genes encoding phospholipid biosynthetic enzymes will be constructed in order to identify the cellular location of these proteins. In order to demonstrate linkage, complementing integrating vectors will be constructed.

Microscopic and biochemical characterisation of phospholipid synthesis mutants. Mutants will be analysed for any defects in cell division by fluorescence microscopy as well as for the presence of phospholipid intermediates by appropriate biochemical tests.

Techniques to be used: Gene cloning, Fluorescence microscopy

References:
(1) Mileykovskaya, E. & Downham, W. (2005). Role of membrane lipids in bacterial division site selection. *Current Opinion in Microbiology*, **8**, 135-142. (2) Bentley, S.D. *et al.* Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2) (2002). *Nature*, 417, 141-147. (3) Bishop, A., Fielding, S., Dyson P.J. & Herron P.R. (2004). Concerted mutagenesis of a streptomycete genome: a link between osmoadaptation, development and antibiotic production. *Genome Research*, **14**, 893-900.

BMS 7 Project Title: Genome sequencing and genome analysis of *Streptomyces rimosus*

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Project Area: (one or more of the following)
Biochemistry/~~Biomedical Analysis/Food Science/Immunology~~/Microbiology/Molecular Biology/~~Parasitology/Pharmacology~~

Project Description:

The bacterium *Streptomyces rimosus* makes the broad-spectrum antibiotic oxytetracycline (OTC). The *S. rimosus* parental strain, G7, makes < 1g/L. However, random strain improvement has generated mutants that can make in excess of 55g/L (1). To determine the genetic basis of this increase in OTC productivity the genome of intermediate OTC-producer, *S. rimosus* 4018, is being sequenced by a mixture of end sequencing and pyrosequencing of a cosmid sub-library. We will then compare this with genome sequences of higher and lower OTC producers – derived later by Solexa sequencing.

OTC production is unstable and known be the result of a >600 kb deletion of the end of the chromosome (2). This project will use such a strain to identify cosmids in our full library that are within this unstable region and then order them. Subsequently, a similar approach will be used to identify cosmids that lie in 'sequencing gaps' that arise during the pyrosequencing of the sub-library. These cosmids will then be sequenced and use to prepare a contiguous *S. rimosus* 4018 genome sequence (3).

The project uses a wide range of molecular biological techniques to address an important commercial problem in the forced evolution of industrially important strains.

Techniques to be used:
Gene cloning, Southern hybridization, DNA sequencing,

References:

1. Petković, H., Cullum, J., Hranueli, D., **Hunter, I.S.,** Perić-Concha, N., Pigac, J., Thamchaipenet, A., Vujaklij, D., and Long, P.F. (2006) Genetics of *Streptomyces rimosus*, the Oxytetracycline Producer. Microbiology and Molecular Biology Reviews **70:**704-728.
2. Pandza *et al.* **(**1997) Physical mapping shows that the unstable oxytetracycline gene cluster of *Streptomyces rimosus* lies close to one end of the linear chromosome. Microbiology **143:**1493–1501.
3. Kirby, R., Gan, T.K., **Hunter, I**, Herron, P, and Tilley, E. (2008) The genome of *Streptomyces rimosus* subsp. *rimosus* shows a novel structure compared to other *Streptomyces* using DNA/DNA microarray analysis. Antonie Van Leeuwenhoek **94:** 173-86.

BMS 8 **Project Title:** Analysis of lipid peroxidation in biological samples

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Project Area: (one or more of the following)
Biochemistry/Biomedical Analysis

Project Description:

Unsaturated lipids are susceptible to oxidative damage by a process called lipid peroxidation, which is important in inflammation and other diseases where reactive oxidants are produced. In view of the importance of this process, a European collaborative action group (COST Action B35) has been set up to study the process in diseases and biological models, and to compare different methods for analysing lipid peroxidation in biological samples. This analytical comparison research will be coordinated by Dr Spickett at Strathclyde, where studies will be carried out using mass spectrometry to analyse oxidized phospholipids, mainly in model lipid and cell samples, but also in samples from animal models of disease.

There is potential for a biochemically-orientated student to be involved in this project, by setting up and testing model systems for lipid peroxidation. This would involve work with purified phospholipids, cholesterol, and cell cultures subjected to different types of oxidative stress, to establish specific protocols that could be shared with other participating laboratories. The analysis would mainly involve electrospray mass spectrometry interfaced with liquid chromatography, but would also utilize other methods of lipid peroxidation analysis for comparison. Depending on achievement, there could be a possibility of an exchange visit to another laboratory in Europe, to analyse samples by alternative methods.

Because of the level of responsibility required and the sophisticated nature of the instrumentation to be used, this project will only be suitable for a student with a biochemistry background and a keen desire to develop advanced technological skills. Please consult Dr Spickett for further information on the project.

Techniques to be used:

Electrospray mass spectrometry and liquid chromatography-mass spectrometry
MALDI mass spectrometry
Cell culture techniques
Treatment of pure lipids or biological samples with oxidants
Extraction of biological samples
Spectrophotometric or fluorescence analysis of lipid peroxidation (POSSIBLY)

References:

1. Direct observation of lipid hydroperoxides in phospholipid vesicles by electrospray mass

spectrometry. SPICKETT, C.M., PITT, A.R. and Brown, A.J. (1998) Free Radic. Biol. Med. 25 (4/5) 613-620.
2. The detection of lipid oxidation in stressed cells by reversed-phase HPLC coupled with positive-ionization electrospray MS. SPICKETT, C.M., Rennie, N., Winter, H., Zambonin, L., Landi, L., Jerlich, A., Schaur, R.J. and PITT, A.R. (2001) Biochem. J. 355 449-457.
3. Studies of phospholipid oxidation by electrospray mass spectrometry: from analysis in cells to biological effects. SPICKETT, C.M. and Dever, G. (2005) Biofactors 24: 17-31.

BMS 9 Project Title: Visualising transcription complexes in the differentiating bacterium *Streptomyces coelicolor* A3(2)

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Secondary Supervisor: Dr Paul R Herron
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Project Area: (one or more of the following)
Microbiology/Molecular Biology

Project Description:

Streptomycetes are among the most complex of bacteria and *Streptomyces coelicolor* is considered the model organism for the actinobacteria, a group of high-GC Gram-positive bacteria with members that are notable both for their industrial importance as antibiotic producers and for their complex lifecycle wich involves the formation of at least three different cell types. The first step in gene expression, transcription is carried out by the structurally conserved RNA polymerases (RNAP), synthesising single stranded mRNA from a double stranded DNA molecule. Whilst in bacteria we know large amounts relating to the transcriptional cycle in specific promoters, we know little about how chromosome organisation and sub-cellular compartmentalisation affect transcription.
This project aims to generate green fluorescent fusions of RNAP sub-units to investigate transcriptional localisation and the dynamics of transcription in *Streptomyces*. *Streptomyces* offers a extraordinary system to study these processes as it grows apically from the tip and through the use of mutants (ΔftsZ) we can uniquely de-couple cell division from growth and development to elucidate underlying mechanisms of transcription factor dynamics.

Techniques to be used:
The project will require the application of cloning, general molecular biology, gene knock-out technologies and fluorescent time-lapse microscopy for completion.

References:
1. Lewis, P J et al., (2008) Transcription factor dynamics. *Microbiology* **154,** 1837-1844.
2. Doherty, G. P., Meredith, D. H. & Lewis, P. J. (2006). Subcellular partitioning of transcription factors in *Bacillus subtilis*. *J Bacteriol* **188**, 4101–4110.
3. Davies, K. M., Dedman, A. J., van Hork, S. & Lewis, P. J. (2005). The NusA : RNA polymerase ratio is increased at sites of rRNA synthesis in *Bacillus subtilis*. *Mol Microbiol* **57**, 366–379.

BMS 10 Project Title: Determining the therapeutic potential of *Capsella bursa-pastoris*

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Project Area: (one or more of the following)
Biomedical Analysis/Immunology/Microbiology/Molecular Biology

Project Description:

Capsella bursa-pastoris, is a Scottish weed that is believed to have anti-inflammatory and anti-bacterial properties. However, this is from anecdotal evidence only and has yet to be proven scientifically. The aim of this project is therefore to determine whether this plant has any therapeutic properties that can be exploited. Crude solvent extracts will be made from leaves and flowering stems. The extracts will tested for cytotoxicity, anti-microbial and anti-inflammatory properties. The extracts exhibiting strong activity will be fractionated by chromatography and individual active compounds isolated and characterised by NMR. Genes and gene pathways associated with the therapeutic activity will also be examined.

Techniques to be used:

Tissue culture, microbiology, solvent extractions, chromatography, NMR, PCR

References:

1. Iannetta P.P.M., Begg, G., Hawes, C., Young M., Russell, J., Squire, G.R. (2007) Variation in *Capsella* (shepherd's purse): an example of intra-specific functional diversity. *Physiologia Plantarum* **129**, 542-554.
2. Jane Wishart, Kim Evans, Theodore Kypraios, Simon White, Simon Preston, Graham Begg, Ian Dryden, Pietro Iannetta (2008) Measuring plant genetic diversity using inter-simple sequence repeats (ISSRs). *Mathematics in the Plant Science Study Group* (*GARnet*). University of Nottingham, 17th – 20th December 2007. **http://cpib.info/measuring_genetic_diversity.pdf**
3. Sucher, N.J., Carles, M.C. (2008) Genome-based approaches to the authentication of medicinal plants. *Planta Med.* **74**, 603-23.

BMS 11 Project Title: Testing of small molecule analogues (SMAs) of the filarial nematode immunomodulator, ES-62 on mast cell effector function

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Project Area: (one or more of the following)
Biochemistry/Immunology/Parasitology/Pharmacology

Project Description:

Abstract: ES-62 is a protein secreted by filarial nematodes. The molecule is unusual in that it contains phosphorylcholine moieties (PC) covalently attached to an *N*-type glycan (1). ES-62 is a potent immunomodulator, being active against a number of different cells of the immune system and the PC moiety is a major contributor to this (2). The net effect of the immunomodulatory properties of ES-62 is to polarise the immune response towards a Th-2/anti-inflammatory phenotype and indeed ES-62 has been shown to be able to reduce the pathology associated with Th-1-associated inflammatory diseases such as arthritis, thereby indicating a therapeutic potential (3). More recently, data have been obtained indicating that ES-62 can inhibit mast cell effector function thereby implying that the molecule has therapeutic potential in asthma (4). However, ES-62 is a large, complex molecule that would be impossible to artificially produce and hence we have recently turned our attention to the synthesis and testing of small molecule derivatives (SMAs) for use as drugs (5). In this project you will test the effect of the SMAs on mast cell effector function in an attempt to find an SMA that mimics the anti-inflammatory effect of ES-62.

Techniques to be used: tissue culture, mast cell growth and maintenance, ELISA, measurement of mast cell products by biochemical assays

References:

1. 1. Houston, K. M. and Harnett, W. (2004) Structure and synthesis of nematode phosphorylcholine-containing glycoconjugates. Parasitology **129**, 655-662.
2. Harnett, W., Harnett, M.M. and Byron, O. (2003) Structural/functional aspects of ES-62 – a secreted immunomodulatory phosphorylcholine-containing filarial nematode glycoprotein. Current Protein & Peptide Science, **4,** 59-71.
3. Harnett, W., McInnes, I.B. and Harnett, M.M. (2004) ES-62, a filarial nematode-derived immunomodulator with anti-inflammatory potential. Immunology Letters **94,** 27-33.
4. Melendez, A. J. et al. (2007) Inhibition of Fce-RI-mediated mast cell responses by ES-62, a product of parasitic filarial nematodes. Nature Medicine 13, 1375-1381.
5. Harnett, W. and Harnett, M. M. (2006) Filarial nematode secreted product ES-62 is an anti-inflammatory agent: therapeutic potential of small molecule derivatives and ES-62 peptide mimetics. Clinical and Experimental Pharmacology and Physiology 33, 511-518.

BMS 12 Project Title: Determination of functional properties of novel compounds that bind to opioid receptors

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Project Area: (one or more of the following)
Pharmacology

Project Description:
An analysis of novel natural products led to predictions of several compounds having affinity for opioid receptors. This has been confirmed by radioligand binding studies, but it is not known whether the compounds activate or block the receptors. This project will answer this question.

Various classical pharmacological preparations including the guinea pig ileum have presynaptic receptors that respond to opiates by reducing the release of neurotransmitters. Such preparations will be used to test the actions of the novel compounds. If they are agonists, they will reduce responses to neural stimulation; if they are antagonists, they will block the actions of opioid receptor agonists.

The results will help to guide the on-going drug discovery project that is seeking novel analgesic agents.

Techniques to be used:
In vitro organ bath techniques: recording effects of electrical stimulation and various agonists. Analysis of mechanism of action through construction of dose-response curves. Graphical and statistical analyses of results.

References:
1. K. Matsumoto *et al.* (2008). Neuropharmaology **55**, 154-165
2. C. Goicoechea *et al.* (2008). Eur. J. Pharmacol. Doi:10.1016/j.ejphar.2008.07.052
3. L.A. Morrone *et al.* (1993). Br. J. Pharmacol. **109**, 48-52

BMS 13 Project Title: *In vitro* methods to detect compounds that reduce ischaemic damage

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Project Area: (one or more of the following)
Biochemistry/Pharmacology

Project Description:
Ischaemic damage to the heart after heart attacks and to the brain after strokes results in death or severe injury to the victim. Various pharmacological strategies are being tested in order to find drugs that can protect or restore function to tissues following ischaemia. The early stage of drug discovery is currently hampered by problems getting reliable and convenient *in vitro* assay systems for testing new compounds. This project will seek to standardise an assay system using cardiac and neuronal cells in tissue culture. Different methods of simulating ischaemia will be tested and several methods for monitoring cell injury and death will be compared.

Techniques to be used:
Cell culture of mammalian cell lines. Manipulations of buffers and culture conditions to produce ischaemia and hypoxia. Radiometric, chemisluminescent, fluorescent and spectrophotometric assays on multi-well plates. Graphical and statistical analyses of results.

References:
1. D.R. Croslan *et al.* (2008). Brain Res. **1210**, 39-47.
2. Y.-S. Jung *et al.* (2006). Eur. J. Pharmacol. **535**, 220-227.
3. G. Agnetti *et al.* (2006). Life Sci. **78**, 264-270.

BMS 14 Project Title: Nanoparticulate formulations of insulin and their analysis

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Project Area: (one or more of the following)
Drug Delivery

Project Description:

Background Subcutaneous or intradermal injection of insulin remains the central treatment of Type 1 diabetes. Non-invasive protein delivery is actively sought but has met with intractable problems, such as the experience with Exubera™ (Pfizer), an inhalable formulation of insulin. Given orally, insulin is enzymatically digested and undergoes first pass metabolism. Oral insulin formulations based on microemulsions show some promise, [1], as do liposomal [2] and solid-particulate formulations [3]. However, the stability of insulin during encapsulation into these systems and controlled release remains problematic and will be addressed in this project proposal.

Aims

To develop insulin-entrapped nanoparticles that will maintain the native peptide backbone conformation and facilitate controlled release *in vitro*. To compare the *in vitro* performance of these nanoparticulates with microemulsion-type formulations of insulin.

Objectives

1. Formulate insulin-entrapped nanoparticles and microemulsions for oral delivery.
2. Characterise the conformation and aggregation of insulin during formulation.
3. Determine the release profile and stability of insulin in the formulations *in vitro*.

Techniques to be used (cf. objectives)

1. Emulsion-solvent extraction formulation techniques, lyophilisation, size distribution analysis and zeta potential measurement.
2. Steady state and real-time fluorescence techniques, liquid-state circular dichroism (microemulsions) and solid-state circular dichroism (nanoparticulates).
3. HPLC assay development.

References:

[1] Çilek A, Çelebi N, Tirnaksiz F, Tay A. A lecithin-based microemulsion of rh-Insulin with aprotinin for oral administration: investigation of hypoglycemic effects in non-diabetic and STZ-induced diabetic rats. *International Journal of Pharmaceutics* 2005, 298, 176-185.

[2] Katayama K, Kato Y, Onishi H, Nagai T, Machida Y. Double liposomes: hypoglycemic effects of liposomal Insulin on normal rats. *Drug Development and Industrial Pharmacy* 2003, 29, 725-731

[3] Ye S, Wang C, Liu X, Tong Z, Ren B, Zeng F. New loading process and release properties of Insulin from polysaccharide microcapsules fabricated through layer-by-layer assembly. *Journal of Controlled Release* 2006, 112, 79-87.

BMS 15 Project Title: Melanin-biopolymer interactions: influence of pH and electrolytes

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Project Area: (one or more of the following)
Drug Delivery

Project Description:

Background Melanin is a ubiquitous pigment in the animal kingdom, existing as a reddish-brown coloured biopolymer *pheomelanin* and the black pigment *eumelanin*. The role of melanin appears to be (i) as protective agent reducing the effect of uv light, (ii) as an antioxidant shortening the life times of highly active radicals and finally (iii) as an absorber of metal cations. Melanin binding of drugs has been shown to significantly alter the pharmacokinetics of applied ocular dosage forms [1]. All basic, hydrophobic materials are bound to some extent and drugs used to treat ocular hypotension such as timolol, pilocarpine and epinephrine are known to have an affinity for melanin binding and have been shown to be less effective in highly pigmented eyes as opposed to those which are less pigmented. The strong binding is affected by pH and electrolytes [2] which prompted the thought that interaction with biodegradable polymers might form the basis of a sustained release mechanism for suitable drugs [3]. In order to characterise the interaction more fully, the influence of biodegradation products on melanin binding will be investigated.

Aims

To quantify the pH and electrolyte effects on synthetic and natural melanin with regard to the binding of a suitable drug.

To investigate models of competitive binding between plasma and melanin as a function of drug concentration, and nature of melanin.

Objectives

1. Examine the characteristics of synthetic and natural melanin binding of brimonidine tartrate as a function of pH and anion concentration.
2. Measure the interaction of melanin and plasma with regard to competitive binding.
3. Quantify the release of brimonidine tartrate in a combined melanin- polylactide combination.

Techniques to be used (cf. objectives)

1. Development of HPLC assays for brimonidine tartrate to quantify binding characteristics.
2. Development of micro pH assays to measure interior pH of gel constructs.
3. Fabrication of biopolymer/synthetic polymer constructs

References:

[1]. Koeberle M.J., Hughes P.M., Skellern G.G. & Wilson C.G. (2006) Pharmacokinetics and disposition of memantine in the arterially perfused bovine eye. Pharm Res 23: 2781-2798

[2]. Koeberle M.J., Hughes, P. M., Skellern , G.G. & Wilson C. G. (2003) Binding of Memantine to Melanin: Influence of Type of Melanin and Characteristics. *Pharm. Res 20: 1702-1709.*

[3]. Burke J & Wilson C.G. (2008). U.S. Patent application in progress.

BMS 16 Project Title: Nasal formulations of poorly soluble compounds

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Project Area:
Drug delivery

Project Description:

The nasal route is increasingly being considered as an alternative to parenteral injection for drugs that for various reasons cannot be administered orally. Interest in nasal drug delivery stems from its patient acceptability, direct systemic absorption, and the potential to overcome problems associated with oral instability, low bioavailability, and unacceptable side effect profiles[1]. A major drawback of the nasal route however, is the rapid mucociliary clearance that exists as a part of the natural defence mechanism of the body. Lyophilised mucoadhesive insert formulations designed to overcome difficulties of administering viscous gels nasally have been shown to increase nasal residence time[2] and achieve prolonged therapeutic plasma levels[3].

A further challenge increasingly encountered in pharmaceutical research is drugs with poor solubility profiles. This project proposes to investigate formulation strategies for the incorporation of poorly soluble compounds into the nasal insert dosage form. Characterisation of the process of hydration will be linked to the solubilisation and release of drugs, and in conjunction with physical characterisation will be used to optimise the performance of the system.

Techniques to be used: Lyophilisation, dynamic vapour sorption, texture analysis, dissolution analysis

References:

1. Illum, L. 2003. Nasal drug delivery – possibilities, problems and solutions. Journal of Controlled Release 87 (1-3) p187-198.
2. Fiona J. McInnes, Bridget O'Mahony, Blythe Lindsay, Janet Band, Clive G. Wilson, Lee Ann Hodges, Howard N.E. Stevens. Nasal residence of insulin containing lyophilised nasal insert formulations, using gamma scintigraphy. European Journal of Pharmaceutical Sciences, 2007, 31, 25-31.
3. Fiona J. McInnes, Panna Thapa, Alan J. Baillie, Peter G. Welling, David G. Watson, Ian Gibson, Andrea Nolan, Howard N.E. Stevens. In-vivo evaluation of nicotine lyophilised nasal insert in sheep. International Journal of Pharmaceutics 304 (1-2) 72-82 (2005).

BMS 17 Project Title: Optimising nasal drug delivery formulations

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Project Area:
Drug delivery

Project Description:

The nasal route is an attractive option for drug delivery of compounds which for some reason are not suitable for oral administration[1]. A major drawback of nasal drug delivery can be the rapid mucociliary clearance rate, reducing the length of time the formulation is in contact with the mucosal surface. The half life of clearance of a substance administered nasally is approximately 15-20 minutes. Lyophilised bioadhesive nasal insert formulations that enable convenient single unit dosing, but re-hydrate in-situ to form mucoadhesive gels, have been used in attempts to overcome this difficulty[2]. In-vivo confirmation of increased nasal residence times[2] mean that such formulations have the potential to enhance drug delivery for a variety of therapeutic applications – e.g. extended release, anti-infectives, anti-emetics, analgesics, peptides etc.

As the lyophilised formulations must hydrate to exert their mucoadhesive effect, the process of water uptake and interaction with mucous will be key to the performance of the system[3]. This project will examine formulation of compounds of interest into lyophilised nasal inserts, and the behaviour of the dosages will be characterised in terms of hydration behaviour, drug release profiles, adhesive performance, and other relevant properties.

Techniques to be used: Lyophilisation, dynamic vapour sorption, texture analysis, dissolution analysis, microscopy

References:

1. Illum, L. 2003. Nasal drug delivery – possibilities, problems and solutions. Journal of Controlled Release Vol 87 (1-3) p187-198.
2. Fiona J. McInnes, Bridget O'Mahony, Blythe Lindsay, Janet Band, Clive G. Wilson, Lee Ann Hodges, Howard N.E. Stevens. Nasal residence of insulin containing lyophilised nasal insert formulations, using gamma scintigraphy. European Journal of Pharmaceutical Sciences, 2007, 31, 25-31.
3. Fiona J. McInnes, Alan J. Baillie, Howard N.E. Stevens. The use of simple dynamic mucosal models and confocal microscopy for the evaluation of lyophilised nasal formulations. Journal of Pharmacy and Pharmacology 59 (2007) 759-767.

BMS 18 Project Title: Exploring the silica condensing activities of silicatein-cathepsin chimeras

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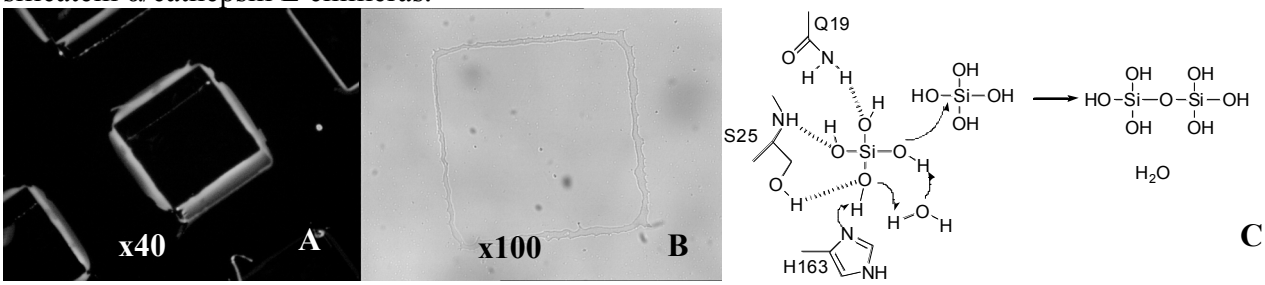
Project Area: (one or more of the following)
Drug Delivery

Project Description:

Background We have engineered a mineral condensing enzyme which can be printed onto a surface, condensing silicic acid and so forming silica patterns, figure below [1]. The enzyme also has the theoretical ability to condense metal oxides. We are now looking to apply this enzyme to drug delivery and biomedical devices, the former investigating the co-encapsulation of proteins from solution and the latter investigating surface adsorption. The project therefore will have several potential areas for exploration. Figure: **A** – fluorescence image of protein stamp; **B**, individual square of silica stamped onto tissue culture plastic; **C**, mechanism for the polymerisation of silicic acid by silicatein α/cathepsin L chimeras.



Aim

To investigate the silica and/or metal oxide condensing abilities of the chimeric enzymes from solution and following surface adsorption

Objectives

1. To determine the growth of the biosilica structures over time and to characterise the surface and internal morphologies of the biosilica.
2. To characterise the localisation of the protein following silica templating in solution and at the surface and investigate the co-entrapment of proteins.
3. To investigate the potential synthesis of composite materials composed of metal oxides using micropatterning approaches and investigate the added functional benefit such composite materials have for microscale medical devices.

Techniques to be used (cf. objectives)

1. Recombinant protein expression in Pichia pastoris and purification techniques
2. Atomic force microscopy with surface etching.
3. Liquid-state and solid-state circular dichroism.
4. Surface plasmon resonance (Biacore™).

References: [1] Fairhead M, Johnson KA, McMahon SA, Kowatz T, Carter LG, Oke M, Liu H, Naismith JH, van der Walle CF, (2008) Crystal structure and silica condensing activities of silicatein–cathepsin L chimeras. Chemical Communications, 1765-1767 (DOI: 10.1039/b718264c)

BMS 19 Project Title: Detection of aggregates of recombinant prion protein using FRET

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Project Area: (one or more of the following)
Biochemistry/ /Immunology/

Project Description:
Changes in the conformation and proteinase resistance of prion proteins are thought to underlie the transmissible spongiform encephalopathies such as CJD in humans, BSE in cows, wasting disease in deer and scrapie in sheep. Despite intensive efforts over the last two decades, the functions of prion protein, the mechanisms of TSE infectivity, the steps in prion protein misfolding pathways and the identity of accessory molecules are not yet defined. Early detection of prion disease also remains a major challenge. To help address these problems we have produced a novel panel of monoclonal antibodies. One of them, mAb P1.1, can recognise disease associated forms of prion protein without the need for proteinase K treatment of tissue samples. It can also distinguish between normal recombinant prion protein peptides and mis-folded, (but non-infectious) aggregated forms.
The aim of this project to see whether antibodies such as mAb P1.1 might be usable in diagnostic assays which utilise Fluorescence Resonance Energy Transfer (FRET) analysis. A small, portable device which enables such assays to be carried out at the point of patient care has also been developed within the University. We would therefore wish to test whether our mAbs can be used in combination with this device to distinguish between normal, monomeric recombinanat prion protein and recombinant prion protein aggregates. If such tests are promising then it may additional be possible to test the system on clinical material.
The student does not need to have direct experience of this field, but will be expected to work diligently to acquire the necessary techniques and to literature background to the project.

Techniques to be used:-
FRET analysis
Western Blotting, gel electrophoresis

References:
1. Michael Jones, Victoria McLoughlin, Katherine Norrby, John G. Connolly, Christine F. Farquhar, Ian R. MacGregor and Mark W. Head (2008)
An antibody to the aggregated synthetic prion protein peptide (PrP106-126) selectively recognises disease-associated prion protein (PrPSc) from human brain
specimens,
Brain Pathology (IF 5.4) ,*Published on-line May2 2008*
e-pub ahead of print: doi:10.1111/j.1750-3639.2008.00181.x
2. Jones M, Head MW, Connolly JG, Farquhar CF, Hornsey VS, Pepper DS, MacGregor IR (2005) Purification of normal cellular prion protein from human platelets and the formation of a high molecular weight prion protein complex following platelet activation. *Biochem Biophys Res Commun.* 2005 Sep 16;335(1):48-56.
3. Gandia J., Lluis C, Ferre S, Franco R and Ciruela F.
Light resonance energy transfer-based methods in the study of G protein-coupled receptor oligomerization.
Bioessays. 2008 Jan;30(1):82-9. Review.

BMS 20 Molecular basis of selective toxicity of antimicrobial minor groove

binders

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Project Area:
BB 940 Biochemistry
BB 941 Microbiology
BB 942 Molecular Biology
MP 903 Drug Delivery

Project Description:
Minor Groove Binders (MGB) are a class of compounds that bind to the minor groove of DNA. By doing so, they can disrupt normal cell processes. Although some MGBs are toxic to mammalian cells and can be used as anticancer drugs, we have identified a class of MGBs that can effectively kill microbial cells, yet which do not display significant toxicity to mammalian cells (1). This makes them ideal for use as antimicrobial agents. However the mechanism of action and the basis for the observed selective toxicity is not known.

The aim of this project is to find out why these compounds can kill bacterial or fungal cells, but cause little damage to mammalian cells. Lines of investigation will include measuring the ability of the compounds to enter cells; to prevent key DNA-dependent processes such as DNA replication and transcription in bacterial and mammalian cells (2); to inhibit topoisomerase activity and supercoiling (3), and perturbation of membrane transport systems.

Techniques to be used:
Fluorescent microscopy
Mammalian and microbial cell culture
Cytotoxicity assays
Toposiomerase, DNA cleavage and supercoiling assays

References:
1. Khalaf AI, Ebrahimabadi AH, Drummond AJ, Anthony NG, Mackay SP, Suckling CJ, Waigh RD. (2004) Synthesis and antimicrobial activity of some netropsin analogues. Org Biomol Chem. 2:3119-27.
2. Gieseg MA, de Bock C, Turner P, Ferguson LR, Denny WA. (2002) The effect of DNA-alkylating agents on gene expression from two integrated reporter genes in a mouse mammary tumor line. Anticancer Drugs. 13:271-80.
3. McHugh MM, Woynarowski JM, Sigmund RD, Beerman TA. (1989). Effect of minor groove binding drugs on mammalian topoisomerase I activity. Biochem Pharmacol. 38:2323-8.

BMS 21 Neuroprotection against oxidative and carbonyl stress

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Project Area:
BB 940 Biochemistry
BB 942 Molecular Biology
59 930 Pharmacology

Project Description:
Oxidative stress has been implicated in a range of neurodegenerative diseases, as well as ischaemia-reperfusion type injury such as stroke. Neuronal cells have limited intrinsic ability to protect themselves against oxidants, and this may lead to cellular damage to lipids, proteins and DNA. Oxidation of lipids leads to the production of lipid peroxidation products, many of which are highly toxic aldehydes (1).

Previously we have shown that the human neuroblastoma cell line SH-SY5Y cell line is extremely sensitive to carbonyl stress (2). We have also shown that treatment of cells with certain phytochemicals can lead to the induction of protective enzymes and that this is sufficient to cause increased protection against reactive aldehydes and oxidants (2).

The SH-SY5Y cell line is neuronal-like and can be induced to differentiate (3,4). What is not known is to what extent differentiated cells have increased levels of protective enzymes and whether or not this can protect them against reactive carbonyls.

In this study, SH-SY5Y cells will be differentiated, and the effect on protective enzyme expression measured using Western blots and RT-PCR. The effect on cell survival will be monitored using MTT assays.

Techniques to be used:
Mammalian cell culture
Cytotoxicity assays
Gene Expression studies

References:
1. Ellis, E.M. 2007 Pharmacol Ther. 115:13-24.
2. Lyon et al., 2008 J Neurochem (in press)
3. Biedler et al., 1978. Cancer Res. 38:3751-7.
4. Monaghan T et al (2008) J. Neurochem. 104, 74-88

BMS 22 Markers of cadmium toxicity in hepatocytes

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Project Area:
BB 940 Biochemistry
BB 942 Molecular Biology
59 930 Pharmacology

Project Description:

Cadmium (Cd) is a highly toxic heavy metal that is encountered as an environmental pollutant, as well as through cigarette smoke and the diet. The mechanism of toxicity is not well understood but in vivo Cd can lead to oxidative stress, inhibit mitochondrial ATP production and induce apoptosis, and can lead to cancer. There is currently no effective treatment.

The main sites of Cd toxicity include lung, liver and kidney. Cd exposure is often long-term, and yet the chronic effects are not well characterized. A previous study has investigated the effect of chronic exposure to cadmium on the expression of marker proteins in the kidney. Exposure to cadmium leads to the overproduction of fibronectin indicating that exposure to Cd can lead to renal fibrosis (1). In the lung, Cd exposure leads to a perturbation of the function of E-cadherin and VE-cadherin (2), which may contribute to the development of pulmonary edema. In liver, long term exposure to Cd is known to cause interstitial fibrosis with minimal cell necrosis and inflammatory cell infiltration. (3).

In this project, we plan to use rat hepatocytes as a model to investigate the effect of Cd exposure on the expression of fibrosis-associated proteins, with the aim of identifying molecular markers that could be used to indicate the level and length of exposure, and also provide valuable endpoints for assessing the effectiveness of treatment.

Techniques to be used:
Mammalian cell culture; Cytotoxicity assays; Gene Expression studies (Western blots; quantitative RT-PCR).

References:

1. Thijssen S, Lambrichts I, Maringwa J, Van Kerkhove E. (2007) Changes in expression of fibrotic markers and histopathological alterations in kidneys of mice chronically exposed to low and high Cd doses. Toxicology. 238:200-10.
2. Pearson CA, Lamar PC, Prozialeck WC. (2003) Effects of cadmium on E-cadherin and VE-cadherin in mouse lung Life Sci. 72:1303-20.
3. Kamiyama T, Miyakawa H, Li JP, Akiba T, Liu JH, Liu J, Marumo F, Sato C. (1995) Effects of one-year cadmium exposure on livers and kidneys and their relation to glutathione levels. Res Commun Mol Pathol Pharmacol. 88:177-86.