## 384P THE EXPRESSION OF DOG HEPATIC CYP2B11, CYP3A12, CYP2C21, CYP2C41 mRNA, PROTEIN AND ENZYMATIC ANALYSIS OF CYP3A4, CYP2C19 AND CYP2C9 SUBSTRATES IN DOG LIVER MICROSOMES

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Interpretation of novel drug exposure and toxicology data from the dog is tempered by our limited molecular and functional knowledge of dog cytochromes P450 (CYPs). The aim of this work was to characterise the expression and function of dog hepatic CYPs.

Total mRNA and/or microsomes were prepared from the livers of 6 male and 6 female Alderley Park beagle dogs (age 7.5 months, wt 7-14 kg), and from 1 macroscopically normal human liver, the latter with the approval of the South Sheffield Research Ethics Committee. CYP mRNA levels were quantified by TaqMan 'Real time' PCR. CYP protein levels were determined by ELISA using Gentest® anti-dog P450 antibodies. The following activities, which are selective markers for individual human CYP isoforms were determined in the same livers: dextromethorphan N-demethylation (CYP3A), warfarin 7'-hydroxylation (CYP2C9), mephenytoin 4'hydroxylation (CYP2C19), and omeprazole 5'-hydroxylation (CYP2C19).

No significant sex differences (unpaired t-test) in the mRNA or protein expression of CYP2B11 (mean±sd mRNA male vs female = 1.65±0.75 vs 2.2±1.3 arbitrary units), CYP3A14 (1.00±0.50 vs 1.00±0.2), CYP2C21 (2.00±0.75 vs 2.75±1.75), or CYP2C41 (0.90±0.45 vs 0.6±0.05) were found.

CYP2C41 mRNA exhibited a polymorphic pattern in the livers studied, with only 5 out of 12 dogs (3 males) expressing this isoform. All livers showed some expression of the 3 other isoforms. The mean relative ratio of CYPs 2B11:3A12:2C21/41 for mRNA expression was 1.6:1:2.8 (male) and 2.3:1:3.5 (female), and for protein expression was 3.0:1:2.3 (male) and 2.8:1:1.7 (female).

The kinetics of dextromethorphan N-demethylation were best described by a two site Michaelis-Menten function. The mean $K_m$ values for the high and low affinity sites were 30 μM and 200 μM, respectively. The former was approximately one order of magnitude higher than that found in human liver. Both male (9.3±1.6 fmol/min/mg) and female (12.3±2.1) dog microsomes showed a very low capacity to 7'-hydroxlate warfarin compared to human liver microsomes (787 fmol/min/mg). The 4'-hydroxylation of mephenytoin in dog liver microsomes (R- >> S-) showed opposite stereoselectivity to that seen in human liver (T. Yosumori, *J. Pharmacol. Exp. Ther.* 283 (1993) 434). The 5'-hydroxylation of omeprazole was best described by a Hill function in three and a single Michaelis-Menten function in a fourth dog (Mean $K_m/K_{50\%}$ = 46 μM, $V_{max}$ = 33 pmol/min/mg). This behaviour differs from that of human data, where 5'-hydroxyomeprazole formation is consistent with two site Michaelis-Menten kinetics. The only significant correlation within the 12 livers studied was that between R-mephenytoin 4'-hydroxylase activity and CYP2B11 mRNA expression ($r_s$ = 0.78, p<0006).

In conclusion, we have identified a distinct pattern of expression of CYP2C41 genes in the Alderley Park beagle and also determined that this strain of dog metabolises human CYP 2C substrates in a manner different to humans. We were not able to identify a functional CYP2C polymorphism using the substrates tested.

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## 385P THE EPHARNET HYPERTENSION TEACHING RESOURCE

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EpharNet (The European Pharmacology Network) was funded from 1998-2001 by the European Commission as a Socrates Thematic Network involving 130 institutions in 28 countries throughout Europe. The objectives of EpharNet are to:

facilitate and maintain communication between European pharmacology teachers;
develop a European dimension to pharmacology teaching;
promote the sharing of teaching resources;
improve the efficiency and quality of teaching;
jointly develop new teaching materials and methods.

There are 2 main outputs from the EpharNet project. First, a web site hosted on www.bps.ac.uk from which are available: examples of pharmacological curricula from across Europe; problem-based learning materials; PowerPoint presentations/ slides; a list of pharmacologically interesting web-sites; a database of the scientific and teaching interests of pharmacologists from across Europe; and a compendium of innovations/good ideas in pharmacology teaching. All this material can be freely used, copied or modified for non-commercial purposes by European pharmacologists.

Second, The EpharNet HYPERTENSION Teaching Resource which can be used as a computer-based learning program and supplied to students for self-directed study but WAS DESIGNED as a Teaching Resource i.e. material which would be integrated into courses by teachers who would provide appropriate guidance and direction on the use of the material by students. The program provides core knowledge at an undergraduate student level though in a considerable number of areas more advanced or detailed material is available (accessed through blue-coloured buttons).

There are the following sections in the teaching resource:
1. Introduction - HYPERTENSION: What is it? How frequent is it? Is it dangerous? What is the danger? Does anti-hypertensive treatment work? Are hypertensives treated satisfactorily?
2. Types of hypertension - primary, secondary and special types.
3. Organs involved - involvement of brain, lungs, heart, eye, arteries and kidney.
4. Decision to treat - factors affecting this decision, algorithm for decision to treat.
5. Treatment - non-pharmacological and pharmacological. Details of drugs used (their structure, indications, mode of action, interactions, dosage, contra-indications, unwanted effects).
6. Clinical cases - two short examples are used.

Each section contains interactive material at different levels of complexity and contains a summary and learning points. A set of multiple choice questions is provided dealing with pharmacological or therapeutic material at easy or average levels of difficulty.

European pharmacologists can obtain a free copy of the CD upon request from their National EpharNet Co-ordinator as detailed on the EpharNet web site. Non-European pharmacologists may, for a small charge, obtain a copy from the British Pharmacological Society.

## 386P A COMPUTER SIMULATION OF EXPERIMENTS TO DEMONSTRATE PHARMACOLOGICAL CONTROL OF GUINEA PIG AIRWAYS

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Computer programs which simulate undergraduate pharmacological experiments are now widely available, and most undergraduate pharmacology courses in the UK employ examples of them.

Here we demonstrate a highly interactive computer simulation designed to demonstrate the action of a number of pharmacological agents and procedures on the lung function of the anaesthetised guinea pig and teach the basic pharmacology of the airways to undergraduate students on courses in which pharmacology is a major component. Data from actual experiments has been used throughout and are based on recordings made using a digital electronic pulmonary monitoring system (PMS: Mumed Ltd, London, UK). This system monitors tracheal airflow and transpulmonary pressure from which a number of lung function parameters may be measured e.g. resistance and dynamic compliance. The actions of a number of mediators and antagonists/inhibitors may be investigated in normal and allergic guinea pigs, the latter having been sensitized by previous administration of ovalbumin.

The program was developed using Macromedia Director (version 7) for PCs (minimum specification: Pentium PC, Windows 95/98/NT4, 16 Mb RAM, 10Mb available HD space 16 bit colour graphics).

The 'Introduction' section combines text and colour graphics and covers: aims and objectives, innervation of airway structures and receptor pharmacology of bronchial smooth muscle, the mechanism of allergen sensitization and includes information and a quiz on the agents and drugs used in the program. 'Methods' describes the animal preparation, and the measurement of airway compliance, resistance and blood pressure. The 'Experiments'section allows the student to select, from a menu, to study the effects of various mediators and inhibitory agents in the normal and allergen-sensitized animals.

Simultaneous traces of resistance, dynamic compliance and blood pressure are presented in a form similar to that in the Mumed recording system and each set of data is accompanied by self-assessment questions which demand interpretation of experimental data presented to them, and an understanding of the underlying control mechanisms. These student-centred activities make the program useful for self-directed learning or, in the ideal situation, it would be incorporated into a structured teaching programme and used with a teacher-designed workbook.

It is envisaged that the program could be used in a number of ways: to better prepare students who will perform the practical at a later date; to debrief students after they have performed the practical; as a 'fallback' to provide data for students whose experiments were unsuccessful; as an alternative to the practical, though it should be remembered that different learning objectives may be achieved.

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## 387P CHEMOKINES AND THEIR DIVERSE BIOLOGICAL PARADIGMS

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Scientific advances continue to identify members of the chemokine supergene families as biologically diverse mediators of important physiologic events. While initial investigations originally defined the biological activity of chemokines as proteins with novel chemotactic activity for specific sub-populations of leukocytes, data now supports a much broader biological role for the chemokines.

The chemotactic activity of chemokines for specific leukocyte sub-populations is, in itself, an important activity, as this response provides a mechanism for the successful delivery of the appropriate leukocyte population from the lumen of the vasculature to a site of inflammation. This biological response provides the means for the accumulation of either granulocytes at foci of acute inflammation, via the activity of CXC chemokines, or the accumulation of mononuclear cells at foci of chronic inflammation, via the activity of CC chemokines.

However, leukocyte chemotaxis may not be the only, or the most important, activity of the chemokine family members. A variety of reports have stressed the key role of chemokines in a variety of physiologic and pathologic situations, which may provide mechanisms for activating cytokine networks, altering the expression of adhesion molecules, increasing cell proliferation, regulating angiogenesis, promoting viral-target cell interactions, haematopoiesis and activating the innate immune system. The importance of chemokines as a contributing player to the immune response is further underscored by recent investigations that have identified viral genes that encode chemokine binding proteins.

Chemokines have also been shown to participate in the progression of chronic inflammation by influencing mononuclear cell chemotaxis, hematopoiesis, angiogenesis, stromal cell proliferation, matrix deposition and lymphocyte polarization. This latter activity is especially important, as specific chemokine ligand/receptor pairs have been identified in type 1 (Th1) versus type 2 (Th2) immune responses. For example, investigations have identified that type 1 responses are promoted by the participation of the following chemokine ligands: MIP-1 alpha, RANTES, IP10, Mig, and ITAC and the chemokine receptors: CCR1, CCR5, CXCR3; while type 2 response are driven in part by the participation of eotaxin, MDC, MCP, TARC, I-309, SDF and the chemokine receptors: CCR2, CCR3, CCR4, CCR8, and CXCR4.

These observations have played an important role in the design of efficacious small molecular weight antagonists to therapeutically target specific chemokine receptors, as these receptors and their ligand pairs are likely to participate in the evolution of chronic immune response.