litis, selective beta cell destruction, insulin deficiency and hyperglycaemia (Mordes et al 1996). Abnormality in the central (thymic) tolerance by clonal deletion of potentially autoreactive cells represents a possible contributory factor to the susceptibility of substrains of the BB rat. The abundance of cells expressing inducible nitric oxide synthase (iNOS), termed nitrergic, is proposed as one mechanism involved in the negative selection of self-reactive thymocytes necessary to initiate T-cell dependent beta cell destruction. Nitrergic cells are deficient in autoimmune-susceptible Lewis rat compared with resistant Fischer and Sprague Dawley strains (Downing et al 1998). We therefore examined nitrergic cell abundance in 2 substrains of BB rat – diabetes resistant non-diabetic (DRnd) and diabetes prone, which was subdivided between pre-diabetic (DPpd) and diabetic (DPdb) phenotypes. Beckman glucose Analyzer measurements of blood confirmed rats as either non-diabetic (glucose $< 200\,\mathrm{mg\,dL^{-1}}$) or diabetic (glucose > 250) for at least 2 consecutive days. Five rats ($\sim$13 weeks old) were used for each group, males and females being pooled since there are no differences in autoimmune susceptibility. Paraformaldehyde-fixed thymi were stored frozen (-20°C) before parallel processing of batches containing all 3 groups for enzyme histochemistry in 100-micron sections. Abundance of medullary nitrergic cells stained positive by NADPH-diaphorase was used as a marker of iNOS. Counts and section surface area measurements were made from glycerol-mounted sections, where tissue shrinkage is less than other studies using dehydration and xylene-based mountant. An observer blind to substrain performed the counts. Mean nitrergic cell abundance (count/mm^2) $\pm$ s.e.m. was 0.798 ± 0.133 for DRnd, 0.506 ± 0.065 for DPpd and 0.117 ± 0.032 for DPdb. One-way analysis of variance with Tukey's follow-up test identified significant difference between both DP groups compared with DRnd and between the pre-diabetic and diabetic groups of the DP substrain ($P < 0.001$). Our results identify an association between the DP genotype and deficiency in nitrergic cell abundance and suggest that the diabetic phenotype exacerbates this deficiency, although the possible effect of gender needs to be further investigated.

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Poster Session 1 – Drug Metabolism

069
Metabolism of ibuprofen: synthesis and chromatographic resolution of 3-hydroxyibuprofen

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The metabolism of (*R,S*)-ibuprofen has been extensively investigated, the two major metabolites being 2-hydroxyibuprofen and carboxyibuprofen (Mills et al 1973). The urinary excretion of these metabolites, both free and conjugated, together with the unchanged drug account for approximately 75% of an oral dose following administration of the racemate. The corresponding 3-hydroxy metabolite, the metabolic precursor of carboxyibuprofen, has been identified as a minor urinary metabolite (Brooks & Gilbert 1974) and reported following incubation of the drug with human liver microsomes (Hamman et al 1997). The formation of 3-hydroxyibuprofen results in the introduction of a second stereogenic centre into the drug and thus four stereoisomeric forms of the metabolite are possible. Investigations into the stereoselectivity of ibuprofen oxidation are hampered due to the lack of stereochemically defined samples of the 3-hydroxy metabolite, the formation of which may partially, or totally, determine the stereochemistry of the major urinary metabolite carboxyibuprofen. To address this problem, we now report the synthesis, chromatographic resolution and partial stereochemical characterisation of this metabolic intermediate. The aryl aldehyde, ethyl 2-(4-formylphenyl)propionate, prepared by reaction of the corresponding 4-chloromethyl derivative with hexamine followed by acid hydrolysis (Sommelet reaction), was treated with the phosphrous ylid obtained by reaction of diethylphosphite with 2-bromopropionic acid. The resulting acrylic acid, 3-(4-(1-ethoxycarbonylethyl)phenyl)-2-methylacrylic acid, was converted to a mixed anhydride on treatment with isobutylchloroformate and reduced with sodium borohydride to yield the corresponding alcohol. Ester hydrolysis, followed by catalytic hydrogenation, palladium on charcoal, yielded the target compound, 3-hydroxyibuprofen. Chromatographic resolution of the four stereoisomers of 3-hydroxyibuprofen was achieved using an amylose tris(3,5-dimethylphenylcarbamate) (Chiralpak AD) chiral stationary phase (CSP) and a mobile phase consisting of hexane:methanol:ethanol (96:3:1% v/v/v), containing trifluoroacetic acid (0.05% v/v), at a flow rate of $1.0\,\mathrm{mL\,min^{-1}}$ with UV detection at 220 nm. Under these conditions the stereoisomers were baseline resolved with retention times of 28.8, 34.1, 53.5 and 72.4 min and resolution factors of 2.33, 6.42 and 3.57. Examination of material isolated by semi-preparative chromatography, using the same CSP, by circular dichroism and comparison of the spectra obtained with those of the enantiomers of ibuprofen, indicated that the first and second eluting analyte pairs possessed *R*- and *S*-stereochemistry in the propionic acid moiety. Thus, we conclude that the faster and later eluting pairs of chromatographic peaks correspond to the *R,R*-/*S,R*- and *R,S*-/*S,S*-diastereoisomeric analytes respectively.

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070
In vitro metabolism of captopril ester prodrugs: experimental and molecular modelling approaches

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Captopril is an orally administered ACE-inhibitor that could benefit from the zero order kinetics afforded by percutaneous absorption. A series of straight chain and branch chain esters prodrugs of captopril has been developed as potential candidates for transdermal delivery. These carboxyl ester prodrugs have similar or higher flux to the parent drug in vitro (Gullick et al 2004; Moss 2004). For bioavailabilty issues, it is necessary to investigate the metabolism of the prodrugs in the skin. In vitro metabolism experiments were undertaken to assess the degree of metabolism that the prodrugs undergo, and to assess if structural changes can influence metabolism. The latter issue was also investigated via molecular modelling/docking approaches. In vitro experiments were adapted from a previously published method (Woolfson et al 1990). This method involved incubation of the ester 37°C with porcine liver esterase. Samples were taken at time-points, at which time hydrolysis was quenched using acetone (to de-nature the enzyme). Samples were then filtered and analysed by LC-MS to determine the concentration of captopril and prodrug at each time-point. Molecular modelling experiments were performed to determine the lowest mean docked energy ($\mathrm{kJ\,mol^{-1}}$) between each prodrug and two individual proteins known to reside in the skin. The proteins used for docking studies were Cytochrome P450 2c1 (1BBC) and RNAse (1OG5). Experimentally, the metabolism of each prodrug appears to follow pseudo-first order kinetics. The plots ln(percentage remaining drug) against time yielded straight lines, from which the rate constants and half-lives were determined. Rate of metabolism reached a peak at the ethyl and propyl prodrugs, and decreased substantially thereafter. Further, the difference in rate of metabolism observed between straight chain and branch chain is not significant, indicating very little change in the rate constant due to adjustments in the alkyl chain, and this is supported by the findings from the molecular modelling experiments. Molecular modelling studies indicated that the lowest mean docked energy decreases as ester chain length increases, from –10 to $-16\,\mathrm{kJ\,mol^{-1}}$. It is predicted that larger increases in chain length will lead to a greater reduction of the docking energy. However, this is not a significant issue with prodrug candidates due to the substantial decrease in aqueous solubility and bioavailability as chain length increases.

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071
Oxidatively modified LDL modulate heme oxygenase-1 and glutathoine levels in human aortic smooth muscle cells in response to oxidative stress via the mitogen activated protein kinases

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Atherosclerotic vascular disease is a major cause of death in the western world and involves retention of oxidised LDL in the vessel wall (Ross 1999), leading to endothelial and smooth muscle cell (SMC) dysfunction. Oxidised LDLs induce antioxidant stress proteins such as heme oxygenase-1 (HO-1) (Siow et al 1999) and elevate levels of the endogenous antioxidant glutathoine (GSH) as an adaptive response to increased oxidative injury. HO-1 catabolises

the pro-oxidant heme to the vasodilator carbon monoxide and antioxidants biliverdin and bilirubin. Dietary antioxidants such as vitamin C can protect against vascular cell dysfunction (Carr et al 2000). We investigated whether vitamin C modulates GSH levels and HO-1 expression in human aortic SMC (HASMC) treated with oxidised LDL and examined the involvement of mitogen-activated protein kinases (MAPK) or PKC on HO-1 induction. HASMC were pre-treated in the absence or presence of vitamin C (100 μM) for 24 h and then treated with native (n), moderately (mod) or highly (ox) oxidised LDL (0–300 μg protein/mL, 0–24 h) in the absence of vitamin C. HO-1 protein expression was determined by western blot analysis and intracellular reduced GSH levels analysed using a fluorescence assay. Bilirubin generation was measured as an index of HO-1 activity. To investigate whether oxidised LDL acts via activation of MAPK pathways, phosphorylation of specific kinases were determined. In addition, HO-1 expression was determined in cells treated with U0126 (1 μM, MEK inhibitor), SB203580 (2 μM, p38MAPK inhibitor) or SP600125 (20 μM, c-jun-NH2-terminal kinase (JNK) inhibitor) before treatment with moderately oxidised (mod) LDL (100 μg protein/mL, 24 h). Expression of HO-1, bilirubin generation and GSH levels were elevated to a greater extent by modLDL, containing high levels of lipid hydroperoxides, compared with oxLDL, but unaffected by nLDL. Pretreatment of SMC with vitamin C or MAPK inhibitors significantly attenuated induction of HO-1 and elevation of GSH levels elicited by modLDL ($P < 0.05$, $n = 3–8$). Phosphorylation of p38MAPK, p42/p44MAPK and JNK were enhanced following acute exposure of SMC to oxLDL. These findings suggest that upregulation of HO-1 and GSH is a protective response to oxidative stress via activation of MAPK and that vitamin C affords cytoprotection by attenuating responses to oxidised LDL and hence supplementation may maintain vascular function in atherogenesis.

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Poster Session 1 – Tissue Engineering

072
Formation of alginate hydrogel scaffolds for tissue engineering by light activated release of calcium from photosensitive liposomes

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Light activated release of calcium entrapped in photosensitive liposomes is proposed as a method of controlled gelation of alginate for use as a cell scaffold. The ability to crosslink alginate via this method is dependent on the efficient entrapment of the calcium crosslinker (Ca^{2+}) and incorporation of a photosensitive lipid within the liposomal bilayer. Four liposome preparation methods producing vesicles of different sizes have been studied to evaluate $CaCl_2$ encapsulation. Triplicate preparations of multilamellar vesicles (MLV), small unilamellar vesicles (SUV), interdidgitation fusion vesicles (IFV) and large unilamellar vesicles (LUV) composed of dipalmatoylphosphotidylcholine (DPPC) 75 mol% and cholesterol (Chol) 25 mol% (total lipid 20 mg) were prepared, encapsulating a 0.2 M $CaCl_2$ solution. Unentrapped $CaCl_2$ was removed from the SUV by passing the liposomes down a short column of sephadex G-25, eluting with iso-osmotic buffered saline (20 mM HEPES, 1 mM EDTA buffer pH 7.4) and collecting fractions close to the void volume. Unentrapped $CaCl_2$ was removed from the MLV, IFV and LUV by washing the liposome suspension by centrifugation and repeating this process until no calcium could be detected in the supernatant. This was determined colorimetrically by using the calcium-sensitive dye arsenazo III, which undergoes a colour change upon binding with Ca^{2+} (Ca – AIII $\lambda_{max} = 656$ nm) (Westhaus & Messersmith 2001). Entrapment of $CaCl_2$ was quantified by measuring absorbance intensity using the arsenazo III assay before and after the liposomes had been ruptured using a 10%Triton X100 solution. MLV, IFV and LUV were sized using a Mastersizer (Malvern Instruments) and SUV using a Zeta Sizer (Brookhaven). The entrapment of $CaCl_2$ results (Table 1) indicate that the IFV have the greatest entrapment of $CaCl_2$ and are therefore more likely to cause gelation when the contents are released into an alginate solution. This was confirmed when each of these $CaCl_2$ entrapped liposome preparations were suspended into a 4% solution of sodium alginate (1 part liposome suspension 4 parts sodium alginate). The liposomes were ruptured by the addition of a few drops of 10% Triton X-100, then heated to 50°C and allowed to cool. Only the IFV-alginate sample formed a gel. In all the other liposome preparations the alginate remained viscous with no sign of crosslinking. The synthesis of the photosensitive phospholipid 1,2-bis(4-(n-butyl)phenylazo-4′-phenylbutyroyl)phosphotidylcholine (Bis-Azo PC) has been achieved following the method of Morgan et al (1985). This lipid has been shown to form stable liposomes in the dark when mixed in small quantities with DPPC and photo induced leakage of entrapped solute from these liposomes when exposed to light ranging from 360–470 nm (Bisby et al 2000). By incorporation of Bis-Azo PC into IFV we aim to enable the "light activated release" of entrapped Ca^{2+} and subsequent crosslinking of alginate in close proximity to the liposomes. This will provide a microstructure with a biologically compatible environment for three-dimensional cell proliferation.

Table 1 Liposome preparation characteristics

Vesicle type	Diameter (μm)	$CaCl_2$ entrapped per 20 mg lipid (mg w/w)
MLV	13 ± 0.8	0.05
SUV	0.15 ± 0.05	0.04
IFV	4.8 ± 0.58	2.69
LUV	9 ± 0.6	0.74

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Poster Session 1 – Pharmacy Education

073
A series of practical exercises allowing iterative development of laboratory skills

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In our experience, MPharm students enter the programme with generally poor practical laboratory skills and little or no experience of using any but the most basic laboratory equipment. As part of our programme, a series of three laboratory exercises used to be undertaken by Level 1 students, with the supposed intention of developing laboratory skills. In the authors' experience what actually happened was that students proved grossly incompetent in the first experiment and then moved onto a completely different experiment with similarly disastrous outcomes, which in turn was followed by a third fruitless exercise. The students gained little other than a sense of personal incompetence. The intention was to design a series of practical exercises with sufficient commonality to allow an iterative process of critical self-appraisal and improvement, while retaining a sense of development. This would then be followed by implementation and observation of the results obtained. Students were set a series of three enzymology experiments, each based upon studying the reaction velocity for the hydrolysis of varying concentrations of para-nitro phenyl phosphate by alkaline phosphatase. The reaction product – para-nitro phenol – is quantitated by colorimetry. In the first experiment, a Lineweaver-Burk plot is used to determine Vmax and Km under control conditions only, in the second the same parameters are determined under control conditions and in the presence of an inhibitor (Inorganic phosphate). The final exercise is identical to the second, but all observations are duplicated. Stages two and three each involve an approximate doubling of the number of tubes the students have to control, relative to the previous stage. In the first two stages, students are provided with complete instructions, but in the third, they have to take responsibility for planning the timing of the procedure. The series of exercises was undertaken by a class numerically dominated by MPharm students, but including a minority of other students. At each stage, student performance is assessed as "Unsatisfactory", "Satisfactory", "Good" or "Excellent". The grading is largely based upon the quality of practical results, but does also take account of the analysis of the data. The target grade is Excellent and this requires results with good linearity (Both the standardisation graph for para-nitro phenol and the Lineweaver-Burk plot) and good duplication of results. It also requires the absence of any major error