MEETING REPORT

OPTIMIZING DRUGS FOR LOCAL DELIVERY

Highlights from the Society for Medicines Research Symposium, held June 11, 2009, at Novartis Institutes for Biomedical Research, Horsham, UK.

by S. Collingwood, R. Lock and M. Searcey

CURRENT APPROACHES AND FUTURE POSSIBILITIES FOR RETINAL DRUG DELIVERY

Dr. Susan Barker (University of East Anglia, UK) delivered the opening lecture, which detailed current approaches and future possibilities for retinal drug delivery. Retinal diseases, such as age-related macular degeneration (AMD) and proliferative diabetic retinopathy, can lead to loss of sight and have a major impact on a patient's lifestyle and independence. Diseases of the eye, particularly retinal, are often hard to treat. The retina is protected by functional biological barriers, such as the cornea, tear flow and blink reflex, all of which make retinal drug delivery challenging. Topical delivery is only really useful for surface treatment, since typically less than 5% of a topically applied dose of drug will reach the posterior segment of the eye. A number of approaches have been tested to try to improve penetration of drug through the cornea/conjunctiva: formulation modifications attempting to increase residence time on the corneal surface; penetration enhancers; ultrasound; and topical contact lenses. Intraocular injections remain one of the most reliable methods for delivering drugs to the retina, although patient acceptability can be an issue.

Antiangiogenics and antivascular endothelial growth factor agents are used in the treatment of wet AMD. Treatment consists of direct injection into the vitreous humor of the eye, which can be painful and often has to be repeated on a monthly or bimonthly basis. Examples of these agents include ranibizumab (Lucentis) and pegaptanib (Macugen). Photodynamic therapy has also been used to treat wet AMD. Verteporfin (Visudyne) is used as a photosensitizer for photodynamic therapy to eliminate the abnormal blood vessels in the eye associated with wet AMD. Verteporfin is given intravenously within 15 minutes before laser treatment.

Retinal drug delivery has advanced with the development of intravitreal implants. Enabling a more sustained duration of drug action and potential site-specific release via a single implant, which could be replaced every couple of years, is a more attractive treatment option than multiple invasive intraocular injections. Retisert™ is a fluocinolone acetonide intravitreal implant for the treatment of chronic noninfectious uveitis. The device is surgically implanted through the sclera and sutured into place. The drug is released locally to the posterior segment of the eye over approximately 30 months.

SurModics is another company providing sustained drug delivery to the eye for the treatment of diseases such as AMD. SurModics' ophthalmic drug delivery options include durable and biodegradable drug-eluting coatings for use on devices such as I-vation™ intravitreal implants; fully biodegradable polymer implants; microparticle injections; and delivery of drugs intravitreally or subretinally. The I-vation™ system, currently the company's lead product, consists of a rigid nonferrous metallic scaffold (screw) coated with a drug-loaded polymer. This is implanted through the sclera, where drug slowly dissolves into the vitreous humor over approximately 2 years. I-vation is currently in trials for triamcinolone delivery for wet AMD.

SUMMARY

An international panel of speakers together with approximately 70 delegates were brought together by The Society for Medicines Research's symposium on Optimising Drugs for Local Delivery, held on June 11, 2009 at the Novartis Institutes for Biomedical Research, Horsham, UK. The focus of the conference was on the delivery of drugs direct to the site of action and the consequences of this delivery route on delivery technologies, formulation science and molecular design.

Dr. Barker went on to describe other retinal drug delivery systems in early stages of development. A 2-cm-long implantable micropump, which is designed to allow the patient to release drug from a refillable reservoir and can be topped up with drug by a surgeon without the necessity of replacing the device, is at the preclinical stage.[1] In addition, thermosetting gels are being investigated. Applied to the outside of the sclera via subconjunctival injection, the drug would diffuse from the gel (liquid at room temperature but semisolid at body temperature) through the sclera into the vitreous humor and then to the retina. This technology is currently being tested in an ex vivo porcine model.

In conclusion, Dr. Barker stated that although advances had been made in the development of ocular-specific drug deliv-

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ery systems, controlled, site-specific drug delivery to the eye remains inherently challenging.

THE DYNAMIC GASTRIC MODEL

Dr. Martin Wickham (Institute of Food Research, Norwich, UK) continued the delivery theme by describing the dynamic gastric model: the first in vitro model to fully simulate gastric motility and function. Dr. Wickham explained that there was an unmet need for this model in the assessment of pharmaceutical dosage form. The model was developed over 14 years and is based upon extended studies of the stomach using various techniques, including echo-planar magnetic resonance imaging. The dynamic gastric model now provides a full simulation of mechanical processes in the natural system. Although it was originally developed to study gastric physiology, the model has potential in the study of the effects of food on the bioavailability of drugs, in the evaluation of modified-release dosage forms and in alcohol–drug interactions. It also has potential in the study of metabolism and site-specific drug delivery.

EZETIMIBE: A SELECTIVE CHOLESTEROL ABSORPTION INHIBITOR

Dr. Duane Burnett (Schering-Plough, USA) described the trials and tribulations of developing the cholesterol absorption inhibitor ezetimibe. He began by placing cardiovascular disease into context as one of the main causes of mortality in the developed world, with approximately 39% of all deaths in the USA in 2001 attributable to this disease. High cholesterol has long been known as a risk factor for coronary heart disease and the absorption of dietary cholesterol is a target for the development of effective cholesterol absorption inhibitors. Although ultimately its real target lay elsewhere, ezetimibe derived from a program targeting the acyl CoA:cholesterol acyltransferase (ACAT) enzyme, a protein that adds a fatty acid ester to cholesterol, thereby aiding its absorption. The ACAT inhibitor program was based around a lead structure SA58-035, which was a simple biarylamide containing a long hydrophobic sidechain. Analogue synthesis focused on rigidification through the use of azetidinones, which were formed initially through reaction of an imine with an ester under basic conditions. This led to the identification of SCH47949

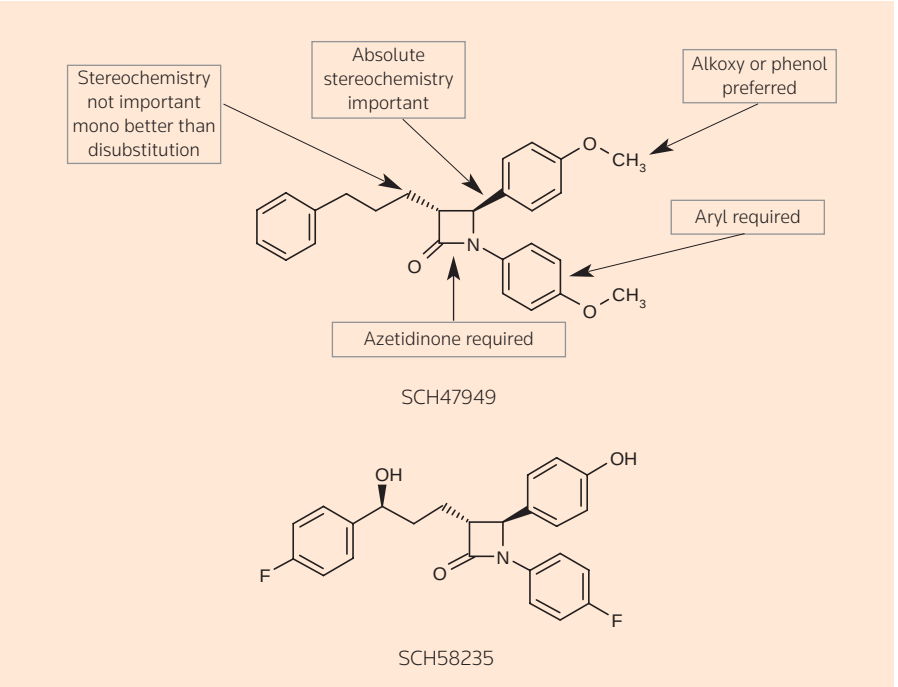


Figure 1.

(Figure 1). The structure–activity relationships in this class of compound were clearly defined; with good activity in biochemical and cellular screens and a good in vivo profile, SCH47949 progressed to phase II trials where it was shown to be well tolerated and effective in humans. It was put on hold in favor of a more interesting backup molecule.

Metabolism studies of SCH47949 demonstrated that key points of metabolism were benzylic hydroxylation, *p*-hydroxylation of the alkylbenzene and demethylation of both methoxy groups. In order to reduce metabolism a new compound, SCH58235, was designed; this proved to be a better clinical candidate than the parent molecule. The only site for metabolism centered on the free phenolic-OH, which is glucoronidated. The resulting enterohepatic recirculation leads to a long half-life of 22 hours. This compound, which was named ezetimibe, does not inhibit or induce cytochrome P450 enzymes, reducing the potential for drug–drug interactions. Marketed in the USA as Zetia and in the UK as Ezetrol, ezetimibe is a selective cholesterol uptake inhibitor that is successful both in monotherapy and in conjunction with statins.

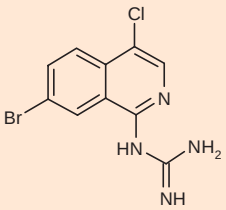
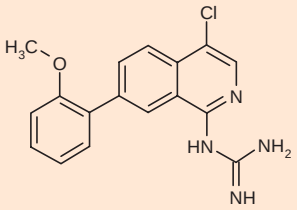
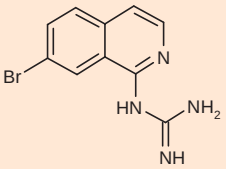
But how does it work? Having been derived from an ACAT inhibitor program, ezetimibe is in fact only a weak inhibitor of this enzyme, suggesting an alternative target. Genomic studies led to the identification of Niemann-Pick C1L1 as a potential cholesterol transporter that represents a likely molecular target of the drug.

OPTIMIZING TOPICAL DRUGS FOR THE TREATMENT OF SCARS AND DERMAL ULCERS

Two discovery phase projects aimed at optimizing topical drugs for the treatment of scars and dermal ulcers were discussed by Dr. Rob Webster (Pfizer, UK).

The first program focused on the discovery of a selective urokinase plasminogen activator (uPA) inhibitor for the treatment of chronic dermal ulcers. uPA is a trypsin-like serine protease that is found in a wide range of cells. It plays a key role in cellular invasion (cancer, tissue remodeling, angiogenesis) and degrades tissue either directly or indirectly through activation of other proteolytic enzymes, initiating a cascade that ultimately leads to matrix degradation. uPA is transiently expressed in normal wounds but consistently high levels are observed in chronic wounds preventing healing. It was proposed that inhibition of uPA would inhibit the proteolytic cascade and thus enable

Table I. *Early 7-aryl isoquinoline series. Potency, lipophilicity and cytotoxicity.*

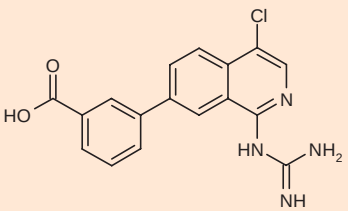
			
K_i (uPA) in nM	290	130	–
clogP ($LogD_{7.4}$)	3.5	4.9 (> 4)	(1.4)
% killed in cytometry	97 (at 10 µM)	96 (at 30 µM)	Not cytotoxic at 100 µM

normal wound healing. Key to the topical drug approach taken was the proposal that the wound surface would have little resistance to drug penetration, while systemic exposure of any topical drug would be limited due to the poor vasculature of wounds. The ideal uPA inhibitor would be potent and selective, stable to a harshly proteolytic environment and rapidly cleared from the systemic circulation. The formulation must be sterilizable and suitable for controlled release.

An early 7-aryl isoquinoline series provided a starting point for the program. A clear correlation between cell cytotoxicity and lipophilicity was quickly established (Table I).

In addition, an X-ray crystal structure of the enzyme and the structure-activity relationship of this series suggested the availability of an acid binding site. A carboxylic acid moiety was introduced to enhance potency and reduce logD, leading to the discovery of UK-356202 (Figure 2).[2]

A pig excisional wound model was developed at Pfizer that consisted of full-thickness excisional wounds (15 mm diameter), in which compound, formulated in Intrasite hydrogel, was applied at a topical dose of 20 mg/cm^2. UK-356202 was delivered at sufficient concentrations to block > 90% uPA activity in the pig model with no reduction in time to heal healthy wounds. UK-356202 was estimated to have a wound half-life of > 2 days; no detectable drug levels were observed in blood or plasma. High unbound clearance was predicted for UK-356202, which was primarily cleared by hepatic uptake, glucuronidation and biliary excretion. A dose of 10 mg/cm^2 was predicted to inhibit uPA in man for up to 7 days.



UK-356202

K_i (uPA) = 17 nM
$logD_{7.4}$ = 2.3
No cytotoxicity at 100 µM
Selective over related enzymes (tissue plasminogen activator, plasmin, thrombin, trypsin). Limited solubility < 100 ng/mL in saline.

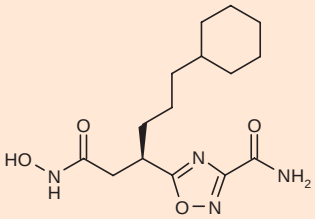
Figure 2.

Results from early clinical studies with UK-356202 were disappointing, with only low levels of compound detected in wound fluid and < 10% median inhibition of ulcer biopsy uPA for all subjects treated with UK-356202 during the study. One hypothesis for the lack of clinical efficacy related to differences between clinical practice and the pig model. In the clinic wound fluid is produced and absorbed into the covering bandages. These bandages contained significant amounts of compound. In contrast, the pig model used a much larger volume of formulation with less opportunity for flow of wound fluid. The clinical batch of drug substance showed lower solubility than that used during the research phase, which may have limited the ability of drug substance to penetrate the wound.

In the second half of his talk Dr. Webster discussed the development of a procollagen C proteinase (PCP) inhibitor for the treatment of dermal scarring. Fibrosis (scarring) is characterized by a buildup of extracellular matrix, in particular Type 1 collagen. PCP plays a key role in producing Type 1 collagen and as such a PCP inhibitor should reduce scar formation. A topical therapy for scarring would need to pass through the epidermis and target the dermis, which is the site of scar formation. Systemic exposure is undesirable and the project team wanted to identify compounds with high intrinsic clearance in vitro and high systemic clearance in rat and dog to minimize systemic exposure following topical application.

A Franz diffusion cell assay was developed to determine transdermal delivery. This allows the measurement of drug absorption through excised human cadaver skin (stratum, corneum, epidermis and dermis). The skin was placed between donor (containing 50% polyethylene glycol) and receiver (containing saline) chambers and the transfer of drug between the 2 chambers was monitored over 24 hours. Compounds with proven transdermal delivery were included as controls.

The project identified UK-383367 as a potential candidate for the treatment of dermal scarring (Figure 3).[3] UK-383367 had a good in vitro profile: appropriate physicochemistry (molecular weight (MWt) 324; $LogD_{7.4}$ 2.6; aqueous solubility in PBS (pH 7.4) 36 mg/mL); potent PCP activity



UK-383367

Figure 3.

(peptide substrate IC_{50} 44 nM; [^{3}H] procollagen IC_{50} 26 nM); good selectivity over the matrix metalloproteinases (> 200-2000 fold); good penetration through human skin; and high in vitro and in vivo clearance in rats and dogs.[4]

In the pig model concentrations of UK-383367 in the dermis following topical application for up to 24 hours were sufficient to inhibit PCP by 70–90%. Despite the presence of a primary amide and the high esterase activity of the epidermis and dermis, UK-383367 had a very long half-life (in the order of days). UK-383367 has not reached the clinic and so translation to the clinical situation is unknown.

DESIGNING DRUGS FOR INHALED DELIVERY TO THE LUNGS

In the first of four lectures describing various aspects of the design of drugs for inhaled delivery to the lungs Dr. Nichola Smith (Novartis, UK) discussed the discovery of a novel class of dimeric epithelial sodium channel (ENaC) blockers for the treatment of cystic fibrosis (CF). In patients with CF the absence of sufficient hydration of the airways renders the natural airway mucus clearance process ineffective and mucous plugs develop leading to bacterial colonization. The prototypical ENaC blocker amiloride has shown equivocal benefit in CF patients;[5] however, it has relatively low potency and a short duration of action following inhaled delivery. The Novartis team wanted to develop novel ENaC blockers with improved potency and reduced side effect potential. The inhaled route of administration provides an opportunity to deliver direct to the target location, as ENaC is expressed in the lung epithelia, thus minimizing systemic side effects and maximizing the therapeutic index (TI). A key mechanism-based side effect of ENaC blockade is elevation of serum potassium (hyperkalemia), resulting from the renal inhibition of ENaC. Achieving an effective blockade of lung ENaC with minimal effect on systemic potassium was a key goal of the project and the focus for development of an appropriate animal model.

The molecular requirements for dry-powder inhalation (DPI) formulations were also discussed. These include high potency, in the low nanomolar range, as the inhalation device technology limits the ideal dose to < 2 mg. High crystallinity (> 125 °C), low hydroscopicity and ideally choice of a salt previously used for the inhalation route all aid the DPIs' feasibility and their ability to be developed. Additional desired properties were low oral bioavailability; high polar surface area (PSA; high MWt/logP) to give low cellular permeability; high metabolic clearance with inactive metabolites; long lung residency to maximize the dosing interval; low solubility; and a slow dissolution rate. Minimizing the potential for hyperkalemia was driven by several considerations: high MWt/low Log D favors biliary excretion; high protein binding to minimize passive renal clearance; and rapid systemic metabolism to inactive metabolites.

Optimization of the amiloride template (Figure 4) led to compounds such as compound A, which had up to 300-fold improved in vitro potency over amiloride.

When dosed as a solution direct to the trachea, compound A attenuated guinea pig tracheal potential difference in a dose-related fashion without concomitant elevation of serum potassium. The lack of effect of compound A on serum potassium may be due to its low renal clearance (0.3% dose eliminated in the urine in 24 hours), which in turn may be derived from its high plasma protein binding (> 99%). Unusual dimeric ENaC blockers were next described, for example compound B, which has a high MWt (731) and PSA (349). Compound B had a similar in vivo profile to compound A in the guinea pig tracheal potential difference model and also lacked effects on serum potassium levels. The ability of such dimeric compounds to enhance mucocillary clearance following inhaled delivery of a DPI formulation to a conscious sheep was also demonstrated for a compound of undisclosed structure.

Dr. Stefan Eirefelt (AstraZeneca, Sweden) provided a drug metabolism and pharmacokinetic perspective on inhaled drug discovery. In particular, Dr. Eirefelt described recent pharmacokinetic efforts to understand the effect of different preclinical formulations and inhalation delivery systems on the deposition and distribution of drug substance in the lung, as well as the impact of absorption kinetics.

For locally acting therapies it was noted that following inhalation it is desirable to have a kinetic profile in lungs that allows for once-daily dosing. A number of strategies have been used to achieve appropriate lung retention and thus the prospect of once-daily dosing. The talk focused on one of

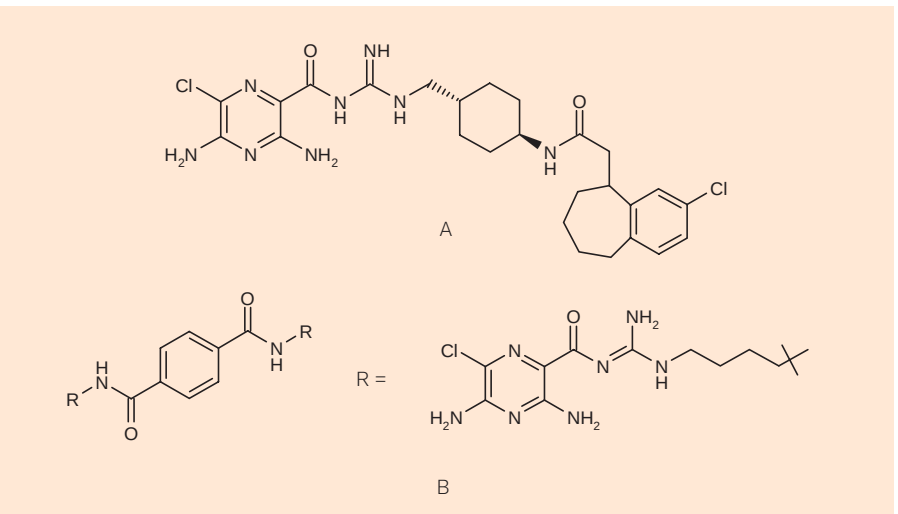


Figure 4.

these strategies, slow dissolution rate, and the in vitro/in vivo methods and challenges to measuring dissolution in preclinical studies.

A number of methods of drug delivery to the lungs have been developed at AstraZeneca. These include intratracheal instillation and small-scale inhalation. From a practical perspective intratracheal instillation of a defined lung dose as a solution or suspension (200 µL) is relatively simple and requires only small amounts of drug substance. However, animals must be anesthetized for dosing and the anesthetic and drug vehicle can affect kinetics. Intratracheal delivery also leads to uneven and central deposition. In comparison, inhalation provides a more even peripheral lung deposition in conscious animals and drug can be delivered as solution, suspension or dry powder. The effect of vehicle is smaller and lung dose is not defined, but calculated or estimated. This method of drug delivery to the lungs is also more clinically relevant. Inhalation studies via nose only (rats) and intubations into the trachea (rats and dogs) were run at AstraZeneca in Sweden to investigate both the pharmacokinetics and pharmacodynamics of potential inhaled therapies.

Dr. Eirefelt described two systems for dry-powder aerosol generation, the Wright Dust Feed and the DustGun (Inhalation Sciences, Sweden). In comparison with the Wright Dust Feed, the DustGun requires smaller amounts of material (~ 5 mg vs. > 500 mg), with a bolus rather than continuous output into the inhalation chamber. The DustGun system consists of: (i) a spray dryer (LaminarPace) for formulating most solutes into manageable powders of micrometer size particles (micronization); and (ii) an aerosol generator to form respirable dry powder aerosols.

Three different exposure models have been linked to the DustGun aerosol generator for screening and selection of drug candidates (see www. inhalation.se):

- (i) an in vitro system (Disolvit) for simulating dissolution and absorption of particles in the lung epithelium
- (ii) an ex vivo rodent-isolated perfused lung system, which is suitable for studying lung specific effects of drugs and toxicants
- (iii) an in vivo system for exposure of laboratory animals or volunteers in preclinical and clinical pharmacology and toxicology, which is under development

A key consideration when characterizing dissolution rates and their impact on lung retention is the use of relevant material and the dosing regime. For example, amorphous vs. crystalline material; crystal polymorph; nonmicronized vs. micronized material; intratracheal administration vs. inhalation in pharmacokinetic and pharmacodynamic studies; and dry-powder inhalation vs. nebulized suspension. Dr. Eirefelt presented a series of examples that showed why it is critical to use relevant material and dosing regime in both pharmacokinetic and pharmacodynamic studies.

In the first example the lung kinetic profile in rats following intratracheal instillation differed between amorphous and crystalline batches of the same compound. The solubility of the crystalline material was far lower than that of the amorphous material and this affected both the lung distribution profile and the percentage of dose remaining in the lung over time. Instillation of crystalline material resulted in a higher exposure in the lung and a longer duration of pharmacokinetic effects.

In the second example the influence of crystalline polymorphs on the lung kinetic profile in rats following inhalation was described. Two polymorphs of the same compound resulted in similar duration in the lung but very different lung concentrations (pmoL/g) and therefore overall lung exposure.

The influence of micronization and particle properties of material on lung levels at 24 hours postdose in rats and mice following intratracheal instillation or inhalation was also discussed. Micronized material generally has a lower mass median diameter than nonmicronized material, resulting in less dose remaining in the lung at a given time point. In addition, inhalation of micronized material compared with intratracheal instillation resulted in a lower percentage of material remaining in the lung. The in vivo pharmacokinetic profile in rat depended on the administration technique, with inhalation leading to lower lung and blood concentrations than an equivalent dose following intratracheal instillation. However, lung duration in this example was not affected by administration technique.

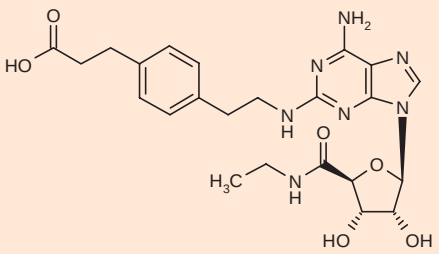
Interestingly, the pharmacokinetic profile of budesonide in dogs following inhalation of dry powder or nebulized suspension did not differ and plasma exposure was the same.

Dr. Eirefelt showed that the in vivo efficacy of budesonide in a Sephadex-induced rat lung edema model differed dramatically depending on administration technique. Budesonide was 10 times more potent after inhalation than after intratracheal instillation. In a tobacco smoke rodent pharmacodynamic model the efficacy of an undisclosed test compound was 1000 times more potent following inhalation than intratracheal instillation.

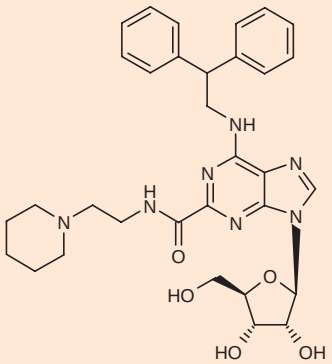
In summary, Dr. Eirefelt stated that while dissolution rate driven lung absorption is one strategy to achieve lung retention with the potential for long duration of action, dissolution rate is only one of several factors that govern lung absorption. There are good in vitro/ex vivo models to predict dissolution in vivo, but it is crucial to use well-characterized drug substance and dosing regime when screening compounds for lung retention and efficacy aimed at once-daily dosing.

In the penultimate lecture of the day Dr. Simon Mantell (Pfizer, UK) described the design of novel inhaled adenosine A_{2A} receptor agonists for the treatment of chronic obstructive pulmonary disease (COPD). Typical symptoms of COPD are cough and lung function decline: this decline may be caused by elevated neutrophil recruitment to the lung, resulting in tissue destruction via superoxide and neutrophil elastase release. Neutrophils express the A_{2A} receptor and its stimulation inhibits release of inflammatory agents. In addition to their anti-inflammatory action A_{2A} receptor agonists are also potent blood pressure-lowering agents, so systemic exposure should be minimized.

The Pfizer team chose CGS-21680 (Figure 5), a potent (IC_{50} 47 nM, inhibitor of superoxide release from human neutrophils) orally available A_{2A} receptor agonist, as a suitable starting point. The first round of optimization led to the discovery of UK-371104, with similar in vitro potency to CGS-21680 but lower liver microsome metabolic stability, which is a desirable property for an inhaled drug.[6] Two in vivo models were used to assess the suitability of newly synthesized compounds. Firstly, they were ranked based on the systemic free maximum concentration (C_{max}) noted after intratracheal dosing in rats (a



CGS-21680
IC_{50} 47 nM
Rat intravenous unbound clearance 82 mL/min/kg
Free C_{max} after inhalation 271 nM
60 min duration in A_{2A} lung model



UK-371104
IC_{50} 65 nM
Rat intravenous unbound clearance 755 mL/min/kg
Free C_{max} after inhalation 20 nM
> 5 hour duration in A_{2A} lung model

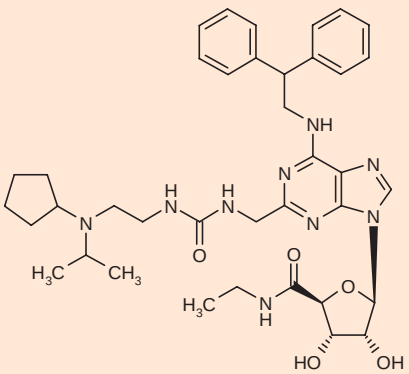
Figure 5.

low C_{max} was preferred). Secondly, the compounds were assessed for the time course of their anti-inflammatory effects and systemic cardiovascular side effect liability in a guinea pig model of capsaicin-induced bronchoconstriction. Intratracheally administered UK-371104 inhibited capsaicin-induced bronchoconstriction in a long-lasting and dose-related manner, with little effect on blood pressure.

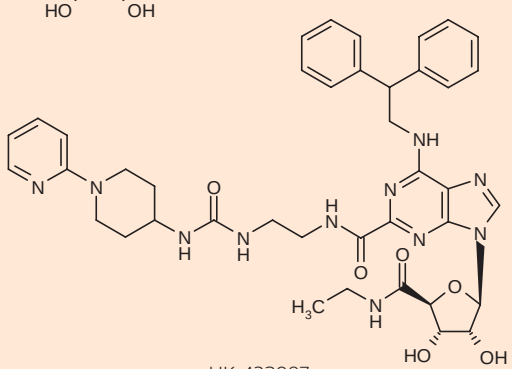
However, the inhaled formulation of UK-371104 was not potent. A further round of optimization resulted in UK-421118 (Figure 6), which had an 8-fold improvement in potency and even higher unbound clearance.[6] The in vitro and in vivo potency of UK-421118 varied depending upon how it was synthesized. This may have been due to its urea group complexing with carbon dioxide in the presence of the terminal basic amine; this occurred during free base preparation via washing salts with carbonate solutions. Concern that this may also occur in vivo led to the removal of the highly basic amino function. The third round of optimization led to the discovery of UK-432097, which did not show any propensity to trap carbon dioxide and had a predicted 250 µg four-times-daily dosing regime in man.[7]

UK-432097 was difficult to crystallize and it was not easy to ascertain which properties of the compound were driving its very low C_{max}; this may have been related to its high MWt and high lipophilicity (Table 2). When dosed as a dry powder via inhalation to healthy volunteers UK-432097 resulted in, as predicted, very low circulating concentrations and did not have any effect on heart rate. A 6-week efficacy study showed no significant improvements in forced expiratory volume in 1 second, a measure of lung function. Several alternative indications are under consideration for UK-432097, including inhibition of cough; lowering of intraocular pressure; improving survival of mechanically ventilated patients; improving wound healing; skin lightening; and treatment of inflammatory bowel disease.

In the final lecture Dr. Mike Woodrow (GlaxoSmithKline, UK) described the optimization of a novel structural class of potent and selective phosphodiesterase



UK-421118
IC_{50} 6 nM
Rat intravenous unbound clearance 3500 mL/min/kg
Free C_{max} after inhalation 1.8 nM
> 5 hour duration in A_{2A} lung model



UK-432097
IC_{50} 4 nM
Rat intravenous unbound clearance 5500 mL/min/kg
Free C_{max} after inhalation 0.2 nM
Clearance process 66% excreted into the bile unchanged, 33% mixture of metabolites
Human recombinant selectivity A_1 300 nM A_{2A} 0.5 nM A_{2B} 68 nM A_3 67 nM

Figure 6.

Table II.

	$A_{2A}IC_{50}$ nM	HLM CL_{int} (µL/min/mg)	Rat CLu (mL/min/kg)	TPSA	logD pH 7.4	MWt	Free C_{max} (nM)
CGS-21680	47	< 8	82	198	-0.15	499	271
UK-371104	65	63	755	158	2.1	602	20
UK-421118	6	135	3500	179	2.0	713	1.8
UK-432097	4	> 255	5500	221	3.4	778	0.2

HLM, human liver microsomes; CL_{int}, intrinsic clearance; CLu, unbound plasma clearance; TPSA, topological polar surface area; MWt, molecular weight; C_{max}, maximum concentration.

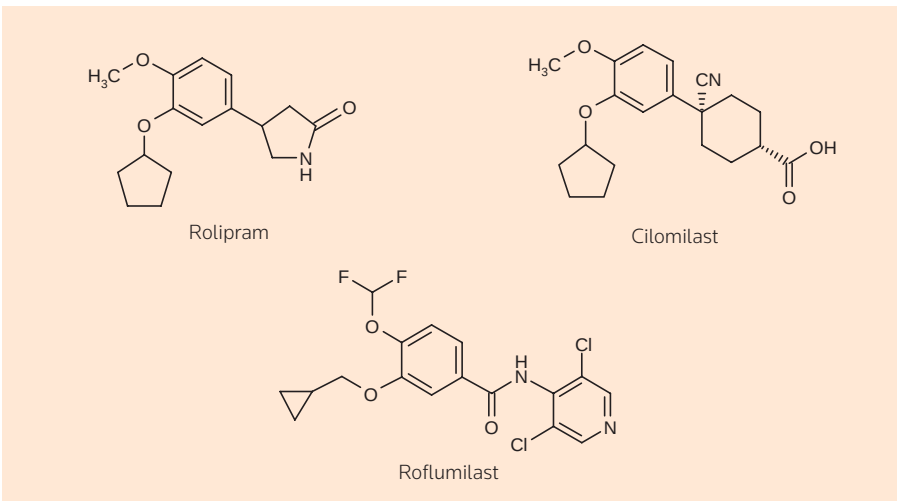


Figure 7.

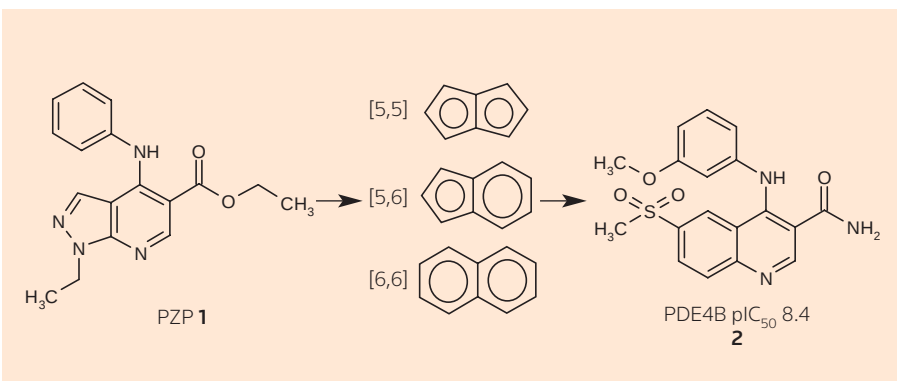


Figure 8.

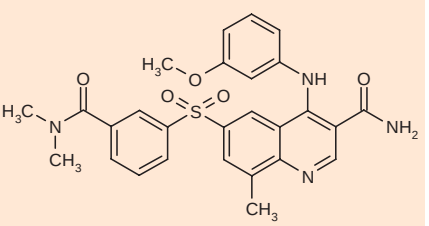
(PDE)4 inhibitors for the inhaled treatment of COPD. Dr. Woodrow gave a brief overview of the role of PDE4 in inflammatory disease and discussed the history of PDE4 inhibitor development. The quintessential first-generation inhibitor rolipram (Figure 7) had pronounced side effects, including nausea and vomiting, at effective anti-inflammatory doses. Second-generation inhibitors followed with an improved TI, for example cilomilast (Ariflo) and roflumilast (Figure 7). GSK have recently begun to look for inhibitors with even greater TIs.

At the initiation of the project the GSK team defined the required compound profile as: high potency at PDE4; good selectivity versus PDEs 1, 2, 3, 5, 6 and 7; and an improved TI (for emesis and vasculitis) over the first- and second-generation compounds. Separate teams investigated oral and inhaled treatments. The inhaled project team further defined their requirements to deliver an improved TI as: high intrinsic metabolic clearance; low systemic exposure; low oral bioavailability; high lung retention (driven by high MWt and high LogP); low dose (to facilitate inhaled formulation development and delivery of a target dose of 50–500 µg); and possibly tight binding to the target (to enhance inhaled duration of action).

Starting from hit pyrazolopyridine (PZP) **1**, two dimensional similarity searching around the PZP template followed by a synthesis cycle generated new hit **2** with good potency at PDE4 (Figure 8).

In addition, **2** displayed good selectivity versus other PDEs, while maintaining cellular potency. It also demonstrated low solubility and low oral bioavailability, which together recommended this chemotype for optimization. Progress was speeded up by the determination of the crystal structure of compound **2** bound to PDE4b. Analysis of the crystal structure suggested two additional binding pockets (close to C4 and the sulphone group of the quinoline) in the cAMP-binding region of the enzyme, potentially suitable for further analogue production. This resulted in the discovery of the highly potent PDE4 inhibitor GSK256066 (Figure 9).

GSK256066 showed excellent potency at PDE4; good selectivity versus the PDE



GSK256066

	pIC_{50}
PDE4B*	≥ 11.5
PDE4A*	≥ 11.3
PDE4C*	≥ 11.4
PDE4D*	≥ 11.9
PDE1	5.8
PDE2	5.9
PDE3	5.9
PDE5	5.5
PDE6	5.3
PDE7	8.4
HARBS	10.3

Figure 9.

selectivity panel; excellent activity in the cellular functional assay; low solubility; and low oral bioavailability (< 1%) in rat. It also displayed a very low dissociation rate from the enzyme. Following inhaled administration of an aqueous suspension in the rat high levels of drug substance were detected in the lung up to 6 hours after dose, with little evidence of systemic exposure in the plasma compartment. When dosed 2 hours prior to challenge in the rat acute lipopolysaccharide-induced pulmonary neutrophilia model GSK256066 inhibited neutrophilia with an effective dose (ED_{50}) of approximately 1.1 µg/kg, which was around 9 times more potent than the control agent fluticasone propionate. GSK256066 was equally effective in the rat ovalbumin-induced pulmonary eosinophilia model. After dosing with GSK256066 0.5 hours prior to and 6 hours after ovalbumin challenge the ED_{50} for reduction in eosinophils, measured 3 days postchallenge, was approximately 0.4 µg/kg compared with fluticasone (33 µg/kg). GSK256066 was selected as a clinical candidate and is currently in phase II clinical trials.

DISCLOSURE

The authors declare no conflicts of interest. The authors are all members of The Society for Medicines Research Committee.

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