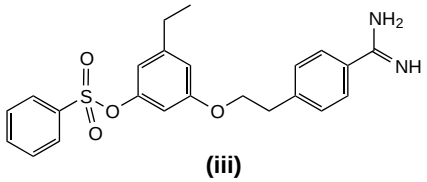
can lead to deep vein thrombosis, myocardial infarction and stroke. The majority of current drug research is focused on finding an antithrombotic drug that is safe and effective and can be administered orally. One prominent target for such a drug is the enzyme thrombin (factor IIa), which is the final key enzyme in the blood coagulation process. It converts soluble fibrinogen to fibrin, which forms the fibrillar matrix of the blood clot. Activation of thrombin initiates several other mechanisms, including both positive and negative feedback of thrombin generation, together with a variety of cellular effects, such as platelet aggregation and tissue remodelling. A low molecular weight substance that can inhibit thrombin, and thus its actions, would be a potentially powerful antithrombotic drug. Finding an inhibitor that possesses both selectivity and suitable pharmacokinetics has been difficult, thus prompting research in this area to continue [4].

A small library of 18 single analogues was prepared in solution. The compounds were analyzed with respect to their inhibition (pIC_{50}) of thrombin, membrane permeability (estimated by migration behaviour in micellar media), pKa and specificity with respect to inhibition (K_i) of trypsin. One of the most potent compounds isolated was **(iii)**, which possessed a pIC_{50} value for thrombin of 7.6. This work has provided a series of compounds worthy of further investigation in the search for a low molecular weight substance that is capable of inhibiting thrombin.



(iii)

4 Linusson, A. *et al.* (2001) Statistical molecular design, parallel synthesis and biological evaluation of a library of thrombin inhibitors. *J. Med. Chem.* 44, 3424–3439

Paul Edwards
Lead Discovery Technologies
Pfizer Global Research and Development
Sandwich
Kent, UK
fax: +44 1304 643555
e-mail: paul_edwards@sandwich.pfizer.com

Drug delivery

Enhancement of pulmonary absorption through the use of mucoadhesive powders

Recently, there has been considerable interest in the pulmonary delivery of drugs with low bioavailability via other routes of administration. The lung as an absorption site has the advantages of low proteolytic activity and an extremely large surface area, which is approximately the size of a tennis court. Several drugs are currently marketed as inhalers, and other drugs are currently in clinical trials as formulations designed to be delivered to the lung. However, the lung is not physiologically designed as an ideal site of absorption. There are mechanisms that function to clear the lungs of foreign bodies, particularly dusts and powders. When attempting to deliver drugs to the lung, these mechanisms, including passive exhalation and mucociliary clearance, which remove dust and powder from the airway, must be confronted.

Mucoadhesive powders have been used successfully in formulations for nasal delivery. Hydroxypropylcellulose (HPC) has been widely used in several pharmaceutical formulations as a mucoadhesive and/or sustained release excipient. As a nasal formulation, coadministration of HPC with the poorly water soluble corticosteroid, beclomethasone dipropionate, enabled the retention time to be prolonged up to 6 h, decreasing the mucociliary clearance rate in the nasal cavity. As a result, the formulation successfully reduced both the daily dose (from 400 to 100 μg day^{-1}) and the dosing frequency (from four times daily to twice daily). To date, the same strategy has not been applied to the formulation of drugs for pulmonary delivery, although the concepts and mechanisms are similar.

Application to pulmonary delivery

Sakagami and co-workers have recently demonstrated the potential application of mucoadhesive powder microspheres to increase pulmonary absorption [1]. HPC microspheres, incorporating fluorescein as a model drug, were prepared and their potential application to enhance pulmonary absorption of drugs was studied. Various formulations of HPC and fluorescein were prepared as microspheres, and the study showed that appropriate formulations increased the bioavailability of fluorescein as a result of both retardation of mucociliary clearance and increased solubility of amorphous, versus crystalline, fluorescein.

The first group of fluorescein microspheres was prepared using HPC-H (an HPC with a very high solution viscosity). Fluorescein was suspended or dissolved in aqueous or ethanol HPC-H solutions and spray-dried. The fluorescein:HPC ratio in aqueous solutions was fixed at 1:4, whereas the ratio in ethanol solutions was varied at 1:1, 1:4 and 1:10. Three other grades of HPC with different solution viscosities were used in a second group of fluorescein microspheres, which were prepared only from ethanol solutions, with a fixed fluorescein:HPC ratio of 1:4.

Scanning electron microscopy (SEM) analysis of the various microspheres showed a nonporous surface and a geometric diameter of 5 μm or less, an easily respirable size. X-ray powder diffractogram analysis of crystalline fluorescein and the various microspheres showed that those prepared from aqueous solutions of fluorescein–HPC-H exhibited peak profiles similar to crystalline fluorescein, indicating that fluorescein's crystallinity was maintained in these microspheres. By contrast, all fluorescein–HPC microspheres

 1359-6446/02/$ – see front matter PII: S1359-6446(02)02205-5

prepared from ethanol solutions exhibited different peak profiles, indicating that fluorescein was incorporated into these microspheres in the amorphous form. The aqueous solubility of fluorescein in the microspheres prepared from aqueous HPC solution was 15.3 μg ml^{-1}, a value comparable to that of crystalline fluorescein (13.5 μg ml^{-1}). By contrast, the aqueous solubility of fluorescein from microspheres prepared from ethanol HPC solutions was approximately 6.5-fold higher, presumably because of the incorporation of amorphous fluorescein. This observation was consistent, regardless of the fluorescein:HPC ratio or the HPC grade used.

Administration in an animal model

The various fluorescein–HPC microsphere formulations were administered to guinea-pigs as powder aerosols: the details of the apparatus are well described by the authors and will not be discussed here. All appropriate control experiments were performed to insure reproducible dose delivery from the inhaler device. The animals were intubated and a 10 min dose delivered from the inhaler. Passively exhaled powders were collected simultaneously. In a second setup (control), the powder aerosols were simply collected. The aerosol chamber was disconnected after dosing but positive pressure was maintained until animals were sacrificed and tissues collected at 0, 60 and 180 min after dosing. Maintaining positive pressure precluded fluorescein loss to the gastrointestinal tract. Comparing the amount that was passively exhaled to the amount collected enabled the calculation of the approximate lung-deposited dose (summarized below).

Despite low aqueous solubility, 61.3 ± 6.4% of the administered dose of 'control' crystalline fluorescein disappeared from the lung within 180 min (the control is non-microsphere crystalline fluorescein). The plasma concentration profiles showed a relatively slower absorption with a T_{max} value of 60 min following administration and the bioavailability (relative to intravenous administration) was calculated to be 45.6%. Various microsphere formulations with a fluorescein:HPC ratio of 1:4 were then tested against the control. Crystalline fluorescein–HPC-H microspheres clearly retarded fluorescein removal from the lung and absorption was reduced compared to the control. Of the administered dose, 74.7 ± 0.7% remained in the lung at 180 min and the calculated bioavailability was only 10.6%, presumably because of their mucoadhesive and/or sustained release characteristics.

By contrast, for the amorphous fluorescein–HPC-H microspheres, >90% of the administered dose was eliminated from the lung at 180 min and the plasma concentration profiles showed rapid absorption with a T_{max} value of 0 min (immediately after administration). As a result, the calculated bioavailability was 88.0% for the microspheres prepared from ethanol solutions. The rapid and extensive absorption was attributed to enhanced dissolution of amorphous fluorescein and HPC's mucoadhesion over the mucociliary clearance. Subsequent experiments exploring the effect of fluorescein–HPC ratio showed that the bioavailability effectively reached a maximum at a fluorescein:HPC ratio of 1:4, and a ratio of 1:1 failed to enhance the extent of absorption.

HPC widely applicable

Amorphous fluorescein microspheres were then formulated from ethanol solutions of four different grades of HPC [super low (SL), low (L), medium (M) and high (H)] of varying solution viscosities: in all cases, the fluorescein:HPC ratio was 1:4. Microspheres formulated with HPC-SL (the least viscous HPC) failed to retard the mucociliary clearance from the lung and fluorescein was transported to the upper tracheo-bronchial airways rapidly (<60 min, based on necropsy of excised lung tissues). By contrast, a significant reduction of fluorescein levels was observed for the microspheres of higher viscosities. This was presumably attributable both to retention of the microspheres in the lung as a result of mucoadhesion, as well as rapid dissolution, release and absorption of amorphous fluorescein from these microspheres. All of these microspheres (with the exception of those made from HPC-SL) showed rapid absorption with a T_{max} value of 0 min because of the enhanced dissolution of amorphous fluorescein, regardless of HPC grade.

The authors appropriately point out that the toxicological and immunological aspects of the inhalation of non-degradable HPC will need to be studied thoroughly before clinical application. However, much higher doses of HPC have not shown any serious side-effects in nasal application (0.6 mg kg^{-1}) compared to the microspheres in this study (0.04 mg kg^{-1} in the case of a fluorescein:HPC ratio of 1:4). These initial results are nonetheless encouraging. Microspheres formulated from ethanol solutions of HPC and fluorescein almost double the bioavailability of fluorescein because of both the effects of mucoadhesive powder retarding mucociliary clearance, as well as better dissolution and absorption of amorphous versus crystalline fluorescein. The formulation could be successful in reducing the therapeutic doses of poorly soluble inhalation drugs and perhaps show wider applications once optimized.

1 Sakagami, M. *et al.* (2001) Enhanced pulmonary absorption following aerosol administration of mucoadhesive powder microspheres. *J. Control. Release* 77, 117–129

John Weidner
Scientist, Parallel Synthesis
Medicinal Chemistry
Emisphere Technologies
765 Old Saw Mill River Rd
Tarrytown, NY 10591, USA
tel: +1 914 785 4792
fax: +1 914 593 8250
e-mail: Jweidner@emisphere.com