Poster Session 2 – Biopharmaceutics

153
A new reflux model for alginate anti-reflux products

F. C. Hampson, A. Banks, I. G. Jolliffe and P. W. Dettmar

Reckitt Benckiser Healthcare (UK), Dansom Lane, Hull, HU8 7DS, UK

Alginate based anti-reflux products are a primary treatment for gastro-oesophageal reflux disease (GORD) in the UK. They provide a physical barrier, in the form of a neutral floating gel or raft, to prevent gastric refluxate reaching the oesophagus. The strength of the barrier has been used as an indicator of in-vitro performance in alginate products. Probe methods have been developed which test raft strength (Washington et al 1986) but there is a need for an in-vitro method which more closely matches physiological reflux. The objective of this study was to develop an in-vitro stomach model which could measure the force required to reflux a raft through a small orifice similar to that presented by the lower oesophageal sphincter during a transient lower oesophageal sphincter relaxation (TLOSR). Alginate rafts were prepared by dispersing the maximum product dose in 0.1 M HCl at 37°C, inside flexible polythene paraboloid shaped bags. These were held in a cylindrical pressure vessel containing water at 37°C. A large volume syringe pump, designed to deliver up to 400 mL of water in 5 s, was connected to the cylindrical vessel so that the 'stomach' contents could be rapidly refluxed, by pressurising and collapsing the flexible polythene bag. The bag contents were forced to reflux through a 1-cm diameter circular hole in a disc shaped probe fitted tightly into the bag. The probe was attached to the arm of a TA-XT2 Texture Analyser and reflux forces were measured with the Texture Analyser set in a stationary but sensitive mode. The reflux resistance of two liquid alginate products, A and B, and a tablet product C was tested and compared with that of a reflux event without product added (Table 1). Each product contained the same maximum dose (1000 mg) of sodium alginate but this was presented as 10% sodium alginate in product A, 5% sodium alginate in product B and two tablets in product C. Statistical analysis, by analysis of variance, showed that rafts formed by product A were significantly more resistant than those formed by product B ($P = 0.035$). Rafts formed by product C were not significantly different from those formed by product B ($P = 0.24$). In all cases the difference between refluxing with a product and refluxing without a product was highly statistically significant ($P < 0.01$). A quantitative in-vitro stomach model has been developed to compare the forces involved in reflux of alginate anti-reflux product rafts. The model can differentiate between products and has clinical relevance because it simulates the in-vivo conditions of a TLOSR, during which reflux is most likely to occur.

Table 1 Raft reflux resistance

Product	Mean force (g)	Max-min force (g)	s.d.
A	1468	1861–816	317
B	1066	1439–910	181
C	808	1052–638	146
None	133	177–86	29

N = 10.

Washington, N., et al (1986) *Int. J. Pharm.* **28**: 139–146

154
Drug degradation in small intestinal fluid in man and the dog

A. S. Carlsson, K. Andersson, K. Björhall*, H. Lennernäs† and B. Abrahamsson

Departments of Biopharmaceutics, *Molecular Sciences, AstraZeneca R&D Mölndal, S-431 83 Mölndal and †Department of Pharmacy, Uppsala University, Box 580, S-751 23 Uppsala, Sweden. E-mail: Anders.S.Carlsson@Astrazeneca.com

The aim of the study was to verify enzymatic-mediated degradation in intestinal fluid of a model drug and to compare the degradation in human intestinal fluid (HIF) and dog intestinal fluid (DIF). Intestinal fluid was collected from fasted subjects by jejunal intubation (man) and mid jejunal fistula (dog), pooled, and after that stored at −70°C. The collection of jejunal intestinal fluid from man was approved by the Ethics Committee of the Medical Faculty, Uppsala University and the Swedish Medical Product Agency, Uppsala, Sweden (Tannergren et al 2003). The degradation of a model drug, AZ1, which included an ester function, was investigated by incubation in intestinal fluid from man and the dog at 37°C. Samples were withdrawn and ice-cold acetonitrile was added to stop all enzymatic reactions. After centrifugation, the drug content and the expected degradation product, the corresponding acid, AZ2, was determined by reversed-phase HPLC with UV detection. Degradation was studied at different drug concentrations, and after adding a mixture of the esterase inhibitors EDTA and phenylmethylsulfonyl fluoride (PMSF). A reference experiment was also performed in a simulated intestinal fluid media (SIF), but without enzymes (Dressman et al 1998). The total amount of proteins was determined, and the proteins in HIF and DIF were separated with a 1-dimentional SDS-PAGE. AZ1 was rapidly degraded in intestinal fluids at the initial phase (the first 5 min) of about 10% per min, and the corresponding acid appeared in a similar rate. The degradation of AZ1, and the formation of AZ2, followed Michaelis–Menten kinetics. No degradation was observed in the SIF without enzymes, and the degradation was inhibited by esterase inhibitors. The V_{max} was higher in the dog than in man (respectively 95 and 22 nmol min^{-1}) and the K_m was 660 and 141 μmol L^{-1} in dog and man, respectively. The total amount of proteins was twice as high in DIF than in HIF; however, the to major protein bands at 27 and 60 kDa, respectively, occoured in both species. In conclusion, enzymatic-mediated degradation in the intestine could be determined by in-vitro testing in real intestinal fluids, and should thus be a useful tool in early drug development. The dog seems to be a reasonably good model for man, although the degradation capacity seems to be somewhat higher possibly due to a higher enzyme concentration.

Dressman, J. B., Amidon, G. L., Reppas, C., et al (1998) *Pharm. Res.* **15**: 11–22
Tannergren, C., Petri, N., Knutson, L., et al (2003) *Clin. Pharmacol. Ther.* **74**: 423–436

155
Selection of a water soluble prodrug for use in the treatment of systemic fungal infections

M. J. Humphrey*, B. Henry and D. McCleverty

Pharmaceutical R&D, Pfizer Global R&D, Sandwich, Kent, CT13 9NJ, UK. E-mail: michael.humphrey@pfizer.com

The treatment of serious systemic fungal infections normally involves administration of antifungal drugs by slow intravenous infusion as the drug formulation may be limited by low drug solubility and/or toleration. The use of water soluble prodrugs to overcome such problems with parenteral formulations has previously been described (Stella 1996). The phosphate ester prodrugs of fluconazole and voriconazole were prepared (Bentley et al 2002) and their water solubility shown to be improved by some 1000 fold to greater than 3 g mL^{-1}. However, the design and selection of a prodrug with improved solubility requires a number of other criteria to be met, both by the prodrug and parent drug, if a successful product is to be developed. Firstly, the prodrug must have good stability in the formulation and, as the primary degradation pathway will be to the parent drug, the parent drug molecule must have appropriate aqueous solubility and stability for the product to have good 'shelf life' stability. Secondly, the prodrug must efficiently liberate in-vivo the parent drug without the generation of any potential new metabolites or safety issues. Initial experiments evaluated the stability over 14 days of the fluconazole and voriconazole prodrugs (44 mg mL^{-1}) in aqueous solutions under conditions of pH 9 and 30°C. The fluconazole prodrug was essentially stable under these conditions, while for voriconazole prodrug there was a significant 13% loss of potency with formation of both parent drug and degradation products. Futhermore, the voriconazole had precipitated in the formulation having exceeded its solubility limit (ca 0.2 mg mL^{-1}). Thus voriconazole prodrug was not deemed suitable for use in a 'ready to use' solution formulation. The formulation stability of fluconazole prodrug, over a range of pH values and temperatures, was investigated to determine the first-order rate constants for acid catalysed hydrolysis to release parent drug. The data were used to predict product shelf-life based on the assumption that the limiting factor was the saturated solubility of fluconazole (4 mg mL^{-1} at 4°C). Stability studies on the prodrug formulation stored under conditions of pH 8.5 and 25°C indicated a shelf-life of 3.1 years. The chemical and metabolic properties of fluconazole (Humphrey et al 1985) and its phosphate prodrug, illustrate the critical success factors required for the successful development of a parenteral dosage form. Fosfluconazole, a water soluble prodrug of Diflucan, has been successfully developed commercially as a new parenteral dosage form to facilitate the bolus administration of high doses (800 mg) for use in the treatment of patients with systemic fungal infections.

Bentley, A., et al (2002) *Org. Proc. Res. Dev.* **6**: 109–112
Humphrey, M., et al (1985) *Antimicrob. Agents Chemother.* **28**: 648–653
Stella, V. J. (1996) *Adv. Drug Deliv. Rev.* **19**: 311–330

156
Optimising the predictive ability of artificial neural networks

A. P. Plumb, R. C. Rowe, P. York and M. Brown*

PROFITS Group, Institute of Pharmaceutical Innovation, University of Bradford, Richmond Road, Bradford BD7 1DP and *Control Systems Centre, Department of Electrical Engineering and Electronics, UMIST, PO Box 80, Manchester M60 1QD, UK. E-mail phil.plumb@astrazeneca.com

Interest in the use of artificial neural networks (ANNs) for the modelling of pharmaceutical formulations has increased during the last decade. Numerous ANN programs are now commercially available. These programs utilize a range of different backpropagation algorithms for model training and, therefore, have the potential to generate a range of models of differing predictive ability from the same data set. Previously, authors have tended to limit ANN optimization to adjustment of the number of hidden layer nodes and have accepted the default settings for the training algorithm and other training parameters (Chen et al 2002). In this study, the effect of varying the training algorithm on the predictive ability of three ANN programs — InForm (v3, Intelligensys), CAD/Chem (v5.1, AI Ware) and the Neural Network Toolbox (v4) of MATLAB© (v6, The MathWorks) — has been compared. An immediate release tablet formulation data set comprising 205 records was used (Bourquin et al 1998). The data set was subdivided into training (50 records), test (50 records) and validation (105 records) sets. A total of 20 algorithms were evaluated, including variants of standard backpropagation algorithms and Bayesian regularization. With the exception of Bayesian regularization, ANN models, containing a single hidden layer of 3 to 12 nodes inclusive, were generated using the attenuated training method (Plumb et al 2002). Bayesian regularization uses no test set. ANN models using this algorithm (3–12 hidden nodes) were trained against the combined training and test sets. None of the ANN architectures studied here could generate an acceptable model for friability or capping. All InForm and CAD/Chem models yielded training gradients greater than 0.5 for the remaining properties. Of the MATLAB models created, only 51 of 120 met this criterion. Prediction of an independent validation set is considered to be the most accurate measure of the predictive ability of a model (Plumb et al 2002). Models that showed acceptable training were assessed by comparing the predicted and observed property values of the validation data. Choice of training algorithm and hidden layer architecture was shown to exert a significant effect on predictive ability. Nevertheless, the most predictive models generated by InForm, CAD/Chem and MATLAB (Table 1) showed no significant differences in their predictive ability. It is concluded that different ANN packages are capable of generating equivalent models provided that both training algorithm and hidden layer architecture are optimized.

Table 1 Training algorithm and hidden layer structure of the most predictive ANN models

ANN	InForm	CAD/Chem		MATLAB
Training algorithm	Standard batch	Accelerated backprop	Gradient descent	Bayesian regul'n
Hidden nodes	8	10	12	8
Prediction R^2	0.5–0.7	0.4–0.7	0.4–0.7	0.6–0.8

Bourquin, J., Schmidli, H., van Hoogevest, P., et al (1998) *Eur. J. Pharm. Sci.* **6**: 287–300
Chen, Y., Jiao, T., McCall, T. W., et al (2002) *Pharm. Dev. Technol.* **7**: 373–379
Plumb, A. P., Rowe, R. C., York, P., et al (2002) *Eur. J. Pharm. Sci.* **16**: 281–288

157
Non-invasive monitoring of lactate by reverse iontophoresis

S. Nixon*, M. B. Delgado-Charro*† and R. H. Guy*†

*School of Pharmacy, University of Geneva, Switzerland, and †Department of Pharmacy and Pharmacology, University of Bath, UK.
E-mail: Begonia.Delgado@pharm.unige.ch

The goal of this research is to apply transdermal reverse iontophoresis for non-invasive therapeutic drug monitoring and clinical chemistry. L-lactate was selected for study because it serves as a metabolic marker in the critically ill patient and as an indicator of performance in sports training. In-vitro iontophoresis experiments used dermatomed pig ear skin. A 0.4-mA current was applied for 5 h via Ag/AgCl electrodes. The subdermal solution consisted of L-lactate (0.5–4.0 mM) in 25 mM Hepes, 133 mM NaCl buffer at pH 7.4. Electrode solutions consisted of 50 mM NaCl. L-lactate was quantified enzymatically. In-vivo experiments were approved by the ethical committee of the University of Geneva. Two glass cells (2 cm^2), containing 50 mM NaCl, were attached to the forearm of 6 healthy subjects. A constant current (0.6 mA) was passed via Ag/AgCl electrodes for 5 h using a commercial power supply. Every 15 min, the entire anodal solution was removed for analysis and the cell refilled. Lactate was assayed by ionic chromatography. Blood levels of lactate were monitored simultaneously with a marketed device (Accusport, Roche) at each sampling interval after the second hour of current. In-vitro, it was found that: the passive flux of lactate across the skin was negligible; a lactate reservoir exists (perhaps not surprisingly) in excised pig skin; and the anodal extraction flux of lactate [$J_{lactate}$] after 2 h (i.e., once the skin reservoir had been emptied) correlated well with the subdermal concentration [$C_{lactate}$], with a relatively constant efficiency of extraction, $J_{lactate}/C_{lactate}$, of 9.4 ($\pm$0.9) μL h^{-1}. Reverse iontophoretic extraction of lactate in-vivo was facile and more efficient than that in-vitro. The initial samples contained very high and variable levels, again indicative of a local reservoir not related to the systemic concentration. After an hour of iontophoresis, however, this reservoir had been emptied, and the subsequent efficiencies of extraction ($J_{lactate}/C_{lactate}$) in 5 out of 6 subjects were relatively stable: 51.3 ($\pm$10.7), 71.9 ($\pm$16.8), 69.3 ($\pm$19.8), 56.9 ($\pm$8.3) and 55.6 ($\pm$9.5) μL h^{-1}. However, towards the end of the experimental period, in two of these subjects, and throughout the study for the final volunteer, extraction efficiencies were significantly higher: 132 ($\pm$31.6), 130 ($\pm$4.7), and 137 ($\pm$27.7) μL h^{-1}. Whether this is because immobilization of the subject's arm for several hours results in increased subdermal lactate levels at the site of iontophoresis, or for another reason (e.g., contamination from sweat), requires further investigation. In conclusion, therefore, while the concept of non-invasive lactate monitoring by reverse iontophoresis is established by this work, considerably more research is necessary to eliminate potential artefacts and to optimize the approach.

Acknowledgements: Funded by the USAMRAA (DAMD17-02-1-0712) and the US National Institutes of Health (EB 001420). The information presented does not necessarily reflect the position or the policy of the US Government, and no official endorsement should be inferred.

158
Does concomitant administration of alginate affect the bioavailability of omeprazole?

P. W. Dettmar, F. C. Hampson, A. Jain*, S. Choubey* , J. Sykes, S. L. Little and T. Baxter

Reckitt Benckiser Healthcare (UK) Ltd, Dansom Lane, Hull HU8 7DS, UK and *Wellquest Research Pvt. Ltd, Wellspring Hospital, Lower Parel, Mumbai 400 013, India

Omeprazole treats gastro-oesophageal reflux disease (GORD) by inhibition of the H^+/K^+ ATPase enzyme system at the secretory surface of the gastric parietal cell to reduce acid secretion. Alginate based reflux suppressants work by forming a low-density raft of near neutral pH which floats on the stomach contents and physically impedes gastro-oesophageal reflux. However, there is limited pharmacokinetic information regarding possible drug interaction between these two types of product, although they may be frequently co-prescribed to improve symptom control in GORD patients. This study was designed to compare the pharmacokinetic parameters obtained for omeprazole after multiple-dose administration of tablets (20 mg, once daily 15 min before breakfast for three days) in the presence or absence of 10% w/v liquid alginate suspension (10 mL, four times daily 30 min after meals). Dosing of each treatment regimen was for 3 consecutive days with a washout period of 7 days between dosing periods. Blood samples for pharmacokinetic analysis were taken over the 24-h period following the final (day 3) dose of omeprazole at 30-min intervals to 4 h, then at 5, 6, 8, 12 and 24 h. Twenty-six male subjects, aged 18–28 years, participated in this randomised, two treatment, two sequence, two period crossover study. The trial protocol was approved by an Independent Ethics Committee and the study was conducted in accordance with local regulatory requirements. Two subjects were withdrawn following the first dosing period and the pharmacokinetic analysis was based on 24 subjects completing the study. Two subjects reported adverse events, one reporting loose motions while on treatment with omeprazole plus alginate (considered possibly related to treatment) and one reporting fever while on treatment with omeprazole only (considered unrelated to treatment). Mean (log transformed)

C_{max} was 558 for omeprazole alone compared with 555 for omeprazole plus alginate. The mean ratio was 99.55% (90% confidence interval 82.75–119.75%). Mean (log transformed) $AUC_{0\text{-}t}$ was 2094 for omeprazole alone compared with 2050 for omeprazole plus alginate. The mean ratio was 97.90% (90% confidence interval 87.83–109.12%). Mean (log transformed) $AUC_{0\text{-}inf}$ was 2231 for omeprazole alone compared with 2247 for omeprazole plus alginate. The mean ratio was 100.74% (90% confidence interval 90.05–112.70%). Mean values for T_{max}, K_{el} and $T_{1/2}$ were also similar for the two treatment regimens. As the 90% confidence intervals for C_{max}, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}inf}$ are all contained within the bioequivalence interval of 80–125%, it can be concluded that the concomitant administration of 10% liquid alginate 10 mL four times daily does not affect the pharmacokinetic profile of omeprazole 20 mg once daily.