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POLYMERIC SYSTEMS FOR SELECTIVE DELIVERY TO THE LARGE BOWEL

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Abstract

A novel disulphide polymer has been synthesised which has been shown to be susceptible to reductive degradation in the colon. The polymer has been coated onto tablets, which have been administered to man. The results from the human testing were extremely encouraging.

A colon-targeted delivery system based on a starch capsule coated with conventional enteric polymers was developed. When this formulation was tested in man, eight out of nine coated capsules disintegrated in the colon. The remaining capsule disintegrated in the terminal ileum.

From studies in rats and a fistulated pig model, an absorption enhancer composition was identified, based on the synergistic effects of two materials. A formulation containing the enhancer and a model peptide encapsulated into a coated starch capsule has been tested in man.

An enteric-coated 5-ASA pellet formulation has been developed. Following extensive in vitro testing, the formulation has progressed to clinical testing in man. Results are awaited.

Introduction

There is a considerable interest within the pharmaceutical industry and research community in the development of pharmaceutical dosage forms which provide targeted delivery of drug into the colon. The availability of reliable colonic targeting systems would be valuable for both the local treatment of large bowel disorders and for the systemic delivery of biopharmaceuticals.

A major part of the BRITE research programme was the development of novel polymers for colon-selective delivery. Polymers were prepared which had elements which would potentially make them susceptible to degradation in the colon. The unique aspect of the colon which can be utilised in this respect is the presence of a large microbial population. Anaerobic bacteria within the colon create a reducing environment. It is possible to prepare polymers which contain within their backbone functional groups susceptible to reductive cleavage. Two such groups are azo (-N=N-) bonds and disulphide (-S-S-) bonds. In the colon, these may be reduced to form $-NH_2$ and -SH groups respectively. Therefore if such functions were included in a polymer backbone, reduction would cleave the polymer chain to produce a lower molecular weight fraction. If the degree of molecular weight reduction is sufficient, the polymer may lose its physical strength or even form water-soluble fractions. The colon-specific degradation of azo-polymers was first reported in 1986 by Saffran and coworkers (Science 233, 1081). The BRITE programme project team speculated that polymers containing disulphide groups may be equally, if not more, susceptible to colonic degradation. The application of disulphide polymers in colon delivery had not previously been reported. It was intended that a novel polymer could eventually be coated onto tablets, capsules or pellets containing drugs for systemic absorption or local action. The other aspects of the research programme were related to developing formulations for these purposes.

Ever since the introduction into medical use of the first therapeutic peptide, insulin, there has been a search for non-injectable routes of delivery, of which the most acceptable to patients is the oral route. It has been proposed that the colon may provide a route of entry into the systemic circulation of large hydrophilic molecules such as peptides and proteins. The rapid growth in the biotechnology industry in the past two decades has generated large numbers of protein and peptide drugs and this has accelerated the interest in the development of effective oral delivery systems for these molecules. However, with peptides and proteins there are considerable obstacles to effective oral absorption because of their large size, their hydrophilic nature and the presence of degradative enzymes in the gastrointestinal tract. It has been suggested that the colon is the most suitable delivery site for oral peptides and proteins because of a lower concentration of digestive enzymes and a lower level of motility than the upper intestine. The lower level of motility leads to the possibility of incorporating excipients into the

dosage form which alter the local colonic environment and thus improve peptide/protein absorption; such excipients might include stabilizing agents or materials which cause a transient increase in the permeability of the colonic epitheliums. As part of this project, a number of potential absorption enhancers were to be screened in animal models for their potential to promote the colonic uptake of insulin. The most successful absorption enhancer formulation would be tested in man. For this purpose, a delivery system would be developed based on conventional pharmaceutical coating materials, since it was anticipated that the novel polymer would not beat a sufficiently advanced stage to be used for this purpose.

The final aspect of the BRITE programme was the development of a pellet based system for delivery of 5-aminosalicylic acid (5-ASA) into the colon. This drug has antiinflammatory activity and is widely used in the treatment of inflammatory bowel diseases (ulcerative colitis and Crohn's disease). Because 5-ASA acts locally in the colon and is well absorbed from the upper intestine, effective therapeutic use requires selective delivery into the colon. This is currently achieved by the use of prodrugs, where 5-ASA is released from the parent molecule within the colon via azo-bond reduction, and by the use of enteric-coated tablets. However, the most widely-used coated tablet preparation, "Asacol", has been reported to have poor reliability in providing colon-specific delivery. Therefore there is clearly a need for more effective formulations to provide delivery of 5-ASA into the colon. Such a formulation could also have application for other drugs which are active in the treatment of inflammatory bowel diseases. Again, this formulation could eventually incorporate the novel colon-specific polymer, but for initial studies would use-a targeting system based on approved pharmaceutical polymers.

Experimental

1 Development of coatings for colon-specific delivery

1.1 Synthesis of polymers

A number of synthetic polymers were prepared by the Laboratory of Organic Chemistry, University of Gent. Polymers were synthesised containing either azo functions or disulphide functions. The following groups of polymers were prepared: i) azo-containing polyamides; ii) disulphide-containing polyamides; iii) disulphide-containing polyurethane; iv) disulphide-containing polyurea/polyurethanes.

1.2 Characterisation of polymers

The polymers were characterised by gel permeation chromatography(GPC) for their molecular weight distribution. The physical behaviour of cast films of the polymers was assessed in terms of elasticity and elongation at break point. The susceptibility of polymer films to degradation in a simulated colonic environment was assessed using two models. The first was

a reductive sulphide-cysteine buffer. The second model was an anaerobic fermenter containing homogenised human faeces suspended in bacterial growth medium.

Selected polymers were tested for their potential as tablet coatings. Preliminary experiments involved some or all of the following: assessment of volubility in pharmaceutically-acceptable solvents; theoretical solubility parameter calculations; glass transition temperature measurements; contact angle measurements. Polymers which had suitable properties were applied to tablets using a small-scale spray-coating apparatus. The resistance of the coated tablets to small intestinal conditions and their sensitivity to colonic environments were assessed using an EP dissolution test, the reducing buffer system, and the bacterial fermenter. The results were used to select the best polymer for in vivo evaluation.

1.3 Toxicity evaluation of selected polymer

From the studies undertaken at Gent and Nottingham, a polymer was selected to be tested in man. Sufficient toxicity data was needed to allow a single administration of the selected novel polymer to man. This requirement would be satisfied by a 14 day sub-acute toxicity study in rats. Groups of rats were dosed with polymer or control (dosing vehicle) and behaviour, food/water intake and body weight were monitored during the dosing period. At the end of the study the rats were sacrificed and tissues and organs examined for signs of toxicity.

1.4 Clinical evaluation of selected polymer

FolIowing toxicity testing, the novel polymer was tested in man in a gamma scintigraphy study.

Tablets were prepared containing lactose and samarium oxide and coated with disulphide polymer to two different thicknesses. The resistance of the tablets to disintegration was assessed in the EP dissolution tester. The tablets were radiolabelled by neutron irradiation to generate the gamma emitting isotope ^{153}Sm. Two groups of eight healthy volunteers received radioactive tablets coated with the two thicknesses of polymer. The passage of the tablets through the gastrointestinal tract and the point of disintegration was assessed externally by gamma camera. .

1.5 Development of a coating system based on approved Pharmaceutical polymers

The research programme also required development of a colon-targeted delivery system based , on approved pharmaceutical polymers in order to undertake early peptide absorption studies in man. From preliminary studies, a promising coating formulation based on enteric polymers was identified. The performance of this coating was assessed in human volunteers. Placebo tablets, hard gelatin capsules and starch capsules which all contained samarium oxide were

coated. Prior to clinical testing, the dissolution performance of the coated dosage forms was assessed using a standard pH change methodology.

The tablets and capsules were radiolabelled by neutron activation and administered to 9 healthy human volunteers in a 3-way crossover study. On each study day, each volunteer received either a coated tablet, a coated gelatin capsule or a coated starch capsule. The passage of the tablets through the gastrointestinal tract and the point of disintegration was assessed externally by gamma camera.

The stability of coated hard gelatin capsules and starch capsules was assessed over a one year period. Size O hard gelatin capsules and size O starch capsules were filled with a powder blend comprising paracetamol and microcrystalline cellulose. The filled gelatin capsules had a gelatin band applied at the capsule cap/body junction. A portion of the capsules were blister packed. Blister packed and non-blister packed capsules were stored at 40 °C/75% relative humidity for 24 weeks or 25 °C/ambient relative humidity for 48 weeks. The dissolution performance of the capsules was tested at 12-week intervals.

2 Enhancement of the colonic absorption of peptide drugs

2.1 Absorption studies in the pig

Following preliminary studies in rats, a fistulated pig model was developed in order to assess the absorption of peptides from the lower intestine of the pig. For oral absorption studies, the pig is considered to represent a good model for man. For the model the pig was surgically modified to create a fistula in the terminal ileum. In addition, the cephalic vein was cannulated to provide a readily-accessible means of long-term blood sampling. A number of formulations containing insulin and absorption enhancers were administered to the fistulated pig. In initial studies the formulations were administered as aqueous solutions into the fistula, but in later studies the insulin and enhancers were administered as tablets or starch capsules. The efficacy of the absorption enhancers was assessed by collecting plasma samples which were analysed for glucose and insulin content.

2.2 Absorption of a model peptide from the gastrointestinal tract of man

A study has been undertaken to assess the oral absorption of a model peptide in man. The peptide has been formulated with an absorption enhancer, identified from the pig studies, and encapsulated in the coated starch capsule system, described in Section 1.5.

3 Development of a multiparticulate (pellet) formulation of 5-aminosalicylic acid for colon-specific delivery

3.1 Compatibility between 5-ASA and formulation components

5-ASA was mixed with a range of excipients. After storage, the mixtures were assayed for incompatibilities by HPLC and differential scanning calorimetry (DSC).

3.2 Preparation of formulations

5-ASA pellet formulations were prepared by coating drug and binder onto sugar seeds using pan coating equipment. The drug-coated seeds were then overcoated with enteric polymers to give the desired dissolution performance.

3.3 In vitro dissolution testing

Two types of dissolution test method were used, namely the EP paddle method and the EP flow-through method.

3.4 Testing of pellet formulations in an in vitro colon fermenter

Two sets of experiments were performed:

a) Incubation of 5-ASA solution in fermenters containing bacterial growth medium, active faecal homogenate or sterile (autoclave) faecal homogenate. Experiments were performed at pH values which represented the healthy colon and the colon in patients with inflammatory bowel diseases.

b) Incubation of pellets under the conditions above. The pellets had been pre-incubated in acid followed by pH 7 buffer.

3.5 Clinical evaluation of pellet formulations

Two 5-ASA formulations were prepared for clinical testing. The pellets were from the same batch, but contained two different levels of coating. Each sub-lot of pellets was tested in eight healthy volunteers. Volunteers were pre-dosed with a radiotelemetry capsule to measure intestinal pH. When the pH recorded indicated that the capsule had emptied from the stomach, the volunteers were dosed with 5-ASA pellets and an indium-labelled transit marker (Amberlite pellets). The movement of the Amberlite pellets was followed by gamma camera imaging. Blood, urine and faeces samples were collected to determine the biodistribution of the drug.

Results and discussion

1 Development of coatings for colon-specific delivery

1.1 Selection of novel polymer

From the synthetic programme and screening tests, a disulphide polymer was selected to be taken into toxicity and clinical testing.

Dissolution test results indicated that the tablets disintegrated approximately 3 times more rapidly in conditions representing the colon. This suggested that the disulphide polymer was a good potential candidate for providing colon-selective delivery.

1.2 Evaluating toxicity of novel polymer

No significant toxicity was observed from administration of the polymer to rats. The duration of the study and the results permitted a single administration to healthy human subjects.

1.3 Clinical evaluation of novel polymer

Results from the clinical study are summarised below:

Tablets with lower level of coating

In five out of eight volunteers, the tablets disintegrated in the colon. In the remaining three volunteers, the tablets did not disintegrate.

Tablets with higher level of coating

In one of the eight volunteers, the tablet disintegrated in the colon. "In the remaining seven volunteers, the tablets did not disintegrate.

Overall, the results were very promising. None of the tablets disintegrated prematurely in the small intestine. The thickness of the coating will require further optimisation to achieve the best colon-selectivity.

1.4 Development of a coating based on approved Pharmaceutical polymers

The dissolution performance of the coated tablets, gelatin capsules and starch capsules was satisfactory, and they were then tested in man.

When tested in man, eight out of nine coated starch capsules disintegrated in the colon, with the remaining capsule disintegrating in the small intestine. For the tablets and hard gelatin capsules,

seven out of nine disintegrated in the colon, the remainder disintegrating in the small intestine.

The stability study on coated starch and gelatin capsules indicated that both were stable for 48 weeks at 25 °C/ambient humidit y. However, at 40 °C/75% relative humidity a significant delay in drug release was found for the gelatin capsules, whereas the dissolution performance of the starch capsules remained unchanged.

2 Enhancement of the colonic absorption of peptide drum

2.1 Absorption studies in the pig

From the absorption studies in the pig, in which a large number of insulin formulations were tested, one absorption enhancer mixture was identified which was particularly promising. The enhancer mixture contained two components, which demonstrated a pronounced synergistic effect. In Figures 1 and 2, plasma glucose vs. time and plasma insulin vs. time curves are presented for this formulation.

2.2 Absorption of peptide when administered orally to man

Results from the assessment of the absorption-enhancing formulation in man were extremely encouraging.

3 Development of a multiparticulate (pellet) formulation of 5-aminosalicylic acid for colon-specific delivery

3.1 Compatibility between 5-ASA and formulation components

Of the materials investigated, only one, shellac, was incompatible with 5-ASA. HPLC analysis of the stored 5-ASA/shellac mixture indicated the presence of 5-ASA degradation products.

3.2 Preparation of formulations

In early batches of pellets, a discoloration was noted after prolonged storage. However, anal ysis failed to identify an y degradation products. The discoloration w as thought to arise from a synthetic impurity in the 5-ASA bulk drug. An extra purification step was introduced by the manufacturer and the problem disappeared.

After several batches at a 1 kg scale had been prepared and tested using a pan coating technique, one was identified which had particularly promising dissolution properties. Further batches were made to confirm that the release profile could be achieved reproducibly at a 10 kg scale . The maximum 5-ASA loading in the pellets achieved using this process method was **63% w/w. ,**

A greater drug loading in the pellets was desirable to achieve a sufficient dose of 5-ASA in a

size O capsule. Therefore a change of process technique to a fluidised bed method was made. The initial results from this technique showed a dissolution profile which matched that found with the pan coated batches, with a 5-ASA loading on the pellets of 70% w/w. A final target specification of 75% w/w 5-ASA w as defined as the required loading, and development work is continuing to achieve this.

3.3 In vitro dissolution testing

The EP flow-through method was found to be more discriminating between batches than the EP paddle dissolution method. With the flow-through method it was found that the release profile was dependent on both the pH profile used and the time course over which the pH was altered. The significance of this finding for in vitro - in vivo correlation is unknown although the relative constancy of the small intestinal transit time in humans may reduce the likelihood of a significant effect.

The dissolution of two pellet batches prepared at 1 kg and 10 kg scales is compared in Figure 3. The dissolution profile was consistent between the two batches at different scales. The variation in profile between sub-batches from the 10 kg lot illustrates how the dissolution profile maybe controlled by modification of the coating thickness.

3.4 Testing of pellet formulations in an in vitro colon fermenter

The degradation experiments showed that 5-ASA was stable in the fermenter contents. However, dissolution of the pellets occurred only in the bacterial growth medium at pH 6.7 and in the active fermenter at pH 4.7. No dissolution was found in the other test conditions.

The pretreatment of the pellets had no influence on release properties.

It is possible that the pellets tested had too great a coating thickness, thus inhibiting release in the fermenters. Therefore coating thickness was investigated in the clinical study.

3.5 Clinical evaluation of pellet formulations

Results from the clinical study are awaited.

Conclusions

There were a number of significant achievements arising from this research programme.

- The development of a synthetic polymer which appears to be susceptible to degradation in the colon is a major advance in the field. Although many studies have been reported in the literature in which azo-polymers have been used for colon delivery, with generally disappointing results, this is the first time in which a disulphide polymer has been prepared and tested for this application. The results from the human testing are extremely encouraging and justify further exploration of the potential of the polymer.

- Although technically less significant, the development of a colon-targeted delivery system based on a starch capsule coated with conventional pharmaceutical excipients is still of great interest. The development of a new synthetic polymer to a stage in which it can be used commercially is a long and expensive process, which presents many challenges. In the interim, there is a need for reliable means of delivery of drugs into the colon. The coated starch capsule forms an ideal delivery system.

- There are limited data in the literature on the oral absorption of peptides in man. In the absence of absorption enhancers, an oral bioavailability of considerably less than 170 might be anticipated. The newly-discovered synergistic mixture of absorption promoting agents shows particular promise. It has been tested in man and yielded encouraging results.

- Finally, the 5-ASA pellet formulation under development by Pharmatec may offer significant benefits over the formulations currently on the market. An important milestone in its development will be successful clinical "testing in man. These results of this pivotal study are expected soon.

Acknowledgments

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Figure 1

Mean plasma glucose concentration following administration of formulation containing insulin and absorption enhancers to pigs

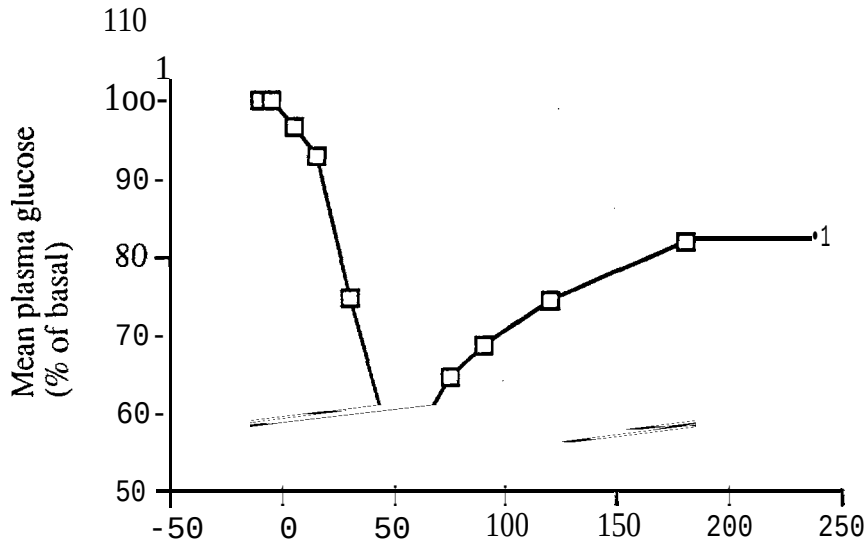

Figure 2

Mean plasma insulin concentration following administration of formulation containing insulin and absorption enhancers to pigs

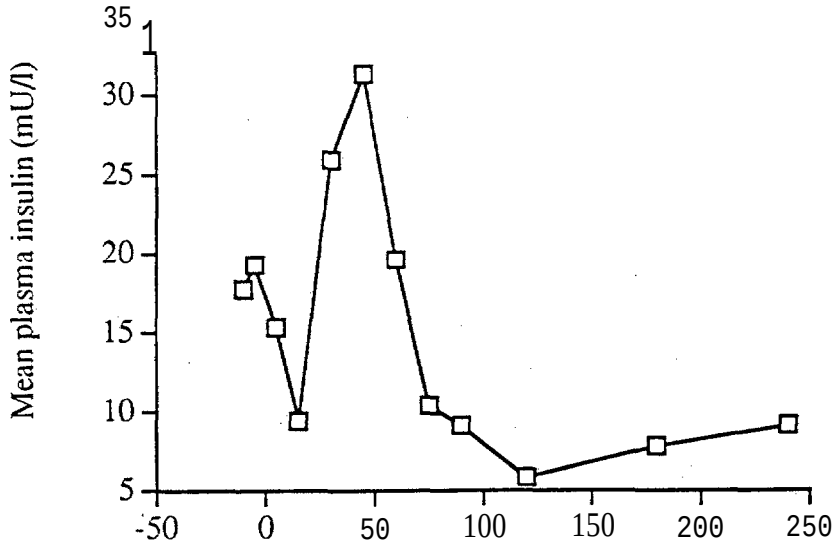

Figure 3
Dissolution profile of batches of 5-ASA pellets in EP flow-through dissolution test apparatus.