The purpose of incorporating peptide drugs into a polymer matrix is to improve the therapeutic efficacy, suitable for parenteral administration to maintain the pharmacological activity for a prolonged period. Many studies have also been attempted to evaluate the polymer matrices. In this study, PLGA microspheres containing salmon calcitonin (sCT) using hydrophilic polymer (RG503H) was much faster compared to hydrophobic polymer (RG503) were prepared by a solvent extraction/evaporation method. Using capillary electrophoresis (CE) and matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS), the in vitro release rates of sCT from two different microspheres were determined, and demonstrated that the polymer properties affected the sCT release pattern from biodegradable PLGA microspheres. Degradation of microsphere and drug release occurred much faster in RG503H polymer than in RG503 polymer. Also, the complex peak between sCT and polymer fragments was detected and increased with the time during the release test. The sCT in RG503 polymer also demonstrated stronger interaction between sCT and polymer units than in RG503H indicating that the hydrophobicity of polymers might be the important factor for the interaction with peptides.

[PE1-24] [04/21/2000 (Fri) 10:30 - 11:30 / [1st Fl, Bldg 3]]

In Vitro Stability of Peptides in Poly(Lactic-co-Glycolic Acid) Microspheres

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One of the critical aspects in the development of peptide/protein-loaded microspheres is the investigation of release characteristics of peptide/protein from microsphere matrix. The stability and chemical changes of peptides in microspheres during in vitro release test were investigated by using capillary electrophoresis (CE) and matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS). Leuprolide and human parathyroid hormone (1-34) (hPTH1-34) were employed as model systems. In the CE electropherogram of two peptides extracted from microspheres after incubation for 3 days at 37oC, the leuprolide microsphere showed no degradation product, while hPTH1-34 microspheres showed the extra peak in addition to the intact peptide. MALDI-TOF mass spectrum of hPTH1-34 extracted from microsphere showed three peaks, including hPTH1-34 (4114.91 m/z) and two peaks (4172.96 and 4228.84 m/z), whereas the leuprolide showed only the mass corresponding to the intact peptide (1208.95 m/z), showing the different stabilities within the microspheres. The two peaks from hPTH1-34 microsphere are corresponded to the association product of hPTH1-34 and polymer fragments. The in vitro release profiles of leuprolide and hPTH1-34 microspheres also showed different patterns. These results indicate that the interaction of peptide to polymer affects the release profiles from the microsphere.

[PE1-25] [04/21/2000 (Fri) 10:30 - 11:30 / [1st Fl, Bldg 3]]

Non-invasive method to encapsulate basic proteins into porous poly(DL-lactide-co-glycolide) microspheres

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Non-invasive method to encapsulate protein into PLGA microspheres has been tried by soaking blank porous PLGA microspheres into protein solution. The incorporation mechanism was systematically studied by varying incorporation parameters such as pH, salt and temperature using five model proteins with different physical properties such as molecular weight and pI. With including NaCl as a porosigen in the primary water phase, porous PLGA microspheres were prepared by W/O/W double emulsion solvent evaporation method using hydrophilic 50:50 PLGA polymer (RG502H, Boehringer Ingelheim) which contains free carboxyl end group. At neutral pH, 37°C, for 24hr, basic proteins, such as lysozyme and ribonulease A were incorporated more than 10% (weight

protein/weight microspheres), whereas acidic proteins such as ovalbumin, bovine serum albumin and human growth hormone were incorporated less than 1%. As the pH of the incorporation medium decreases, incorporation capacity was also decreased. Ionic strength and temperature of the incorporation media were also determined as critical factors of the incorporation capacity. These results suggest that the incorporation is mainly caused by ionic interaction between free carboxyl group of the polymer and positive charges of the basic proteins. Conclusively, this non-invasive method of encapsulation of protein into biodegradable PLGA polymeric matrix can be successfully applied to basic proteins with high drug loading.

[PE1-26] [04/21/2000 (Fri) 10:30 - 11:30 / [1st Fl, Bldg 3]]

THE INTESTINAL ABSORPTION OF HEPARIN DISACCHARIDE USING SEVERAL ABSORPTION ENHANCERS IN CACO-2 MONOLAYERS

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The effect of several absorption enhancers was studied using the Caco-2 cell monolayers on the intestinal absorption of heparin disaccharide, a repeating unit of heparin of which the structure is highly charged and heterogeneous. The absorption enhancing activity of a series of compounds was determined by the changes in transepithelial resistance(TEER) and the transport amount of heparin disaccharide across the Caco-2 cell monolayer by HPLC using SAX column. Among the absorption enhancers, dipotassium glycyrrhizinate, 18β- glycyrrhetinic acid, caprate and taurine decreased TEER and increased the permeability of heparin disaccharide in a dose- and time-dependent manner without severe cytotoxicity. Dibutyryl adenosine 3',5'-cyclic monophosphate, which is an endogeneous cAMP analogue, decreased TEER and increased the transport of heparin disaccharide by almost 10% for control without any toxicity. The combination of sodium deoxycholate and dipotassium glycyrrhizinate or 18β- glycyrrhetinic acid or taurine made the absorption more effective than they were used alone. Our results indicate that these absorption enhancers can widen the tight junction, which is a paracellular absorption route of the hydrophilic compounds, such as heparin, chondroitin sulfate and protein drugs. The oral administration of heparin as well as heparin oligosaccharides may be possible using the selective enhancers together.

[PE1-27] [04/21/2000 (Fri) 10:30 - 11:30 / [1st Fl, Bldg 3]]

Preparation and evaluation of microspheres containing GFP for oral vaccine delivery system

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In order to design the oral vaccine delivery system, we prepared the alginate microspheres containing GFP (Green Fluorescent Protein) as a model drug by spraying method.
To optimize the preperation conditions of microspheres, we investigated the effects of various parameters including nozzle pressure, nozzle pening angle, and concentrations of sodium alginate and calcium chloride . The prepared microspheres were evaluated by measuring their size and loading efficiency, and morphology.
The particle size of microspheres was affected by the concentration of sodium alginate and calcium chloride, and nozzle opening angle, and nozzle diameter. As the concentration of sodium alginate increased, GFP loading efficiency and particles size of microsphere also increased. But more than 1.5%(w/v) sodium alginate solution too viscous, So it was difficult to spary the solution, and particles shape was not spherical.The pressure over 2kgf/cm2 didn't affect the size of particles.
As a result,the spraying method enabled us to prepare microspheres for oral vaccine delivery