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Covalent attachment of polyethylene glycol (PEG) to proteins (PEGylation) is a procedure of growing interest for enhancing the therapeutic potential of protein pharmaceuticals. The PEGylation of recombinant human epidermal growth factor (rhEGF) as method to increase the stability was examined. The PEGylated rhEGF was prepared with succinimidyl propionate(SPA)-monomethoxy-PEG (SPA-mPEG, M.W. 20kD). The mono-PEGylated rhEGF was purified by size-exclusion chromatography and characterized by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Thermal stability test was performed in PBS (10 mM, pH 7.4) at 70oC. Radioiodination of native rhEGF and mono-PEGylated rhEGF was performed with IODO-GEN method. The 125I-rhEGF and 125I-mono-PEGylated rhEGF were mixed with wound homogenate of rat skin and incubated at 36.5℃. Column-switching HPLC method using flow-through radioisotope detector (FTRD) was used for direct analysis of homogenate samples. In thermal stability test, native rhEGF remained 19% after incubation of 39 hours and was not detected after 64 hours, while mono-PEGylated rhEGF still remained 62% after incubation of 64 hours. After incubation in wound homogenate of rat skin for 7 hours, the remained amount of rhEGF and mono-PEGylated rhEGF were measured 37% and 80%, respectively. The degradation peak of rhEGF was detected in FTRD-HPLC chromatogram and the peak was increased with incubation time. Mono-PEGylated rhEGF did not show the degradation peak. In conclusion, this study indicates that PEGylation of rhEGF can improve its stability.

[PE1-21] [04/19/2001 (Thr) 15:30 - 16:30 / Hall 4]

Preparation and in vitro release of LHRH agonist containing poly(d, l-lactide-co-glycolide) microspheres

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Triptorelin, goserelin, and leuprolide are luteinizing hormone-releasing hormone (LHRH) agonists widely used for the treatment of prostate cancer and endometriosis. A mixed oil in water solvent extraction/evaporation method with exactly same manufacturing parameters was employed to fabricate LHRH-agonists containing microspheres using 50:50 poly(d,l-lactide-co-glycolide) (PLGA). Encapsulation efficiency, yield, and size distribution were similar. SEM observation also showed similar internal and external morphologies. However, in vitro release test (33 mM phosphate buffer, pH 7.0 at 37°C), showed quite different profiles. Release rate of leuprolide was fastest and that of triptorelin was slowest. Further extensive studies including in vitro release tests in various different conditions and in vivo release efficacy should be followed to correlate well the in vitro-in vivo release profiles.

[PE1-22] [04/19/2001 (Thr) 15:30 - 16:30 / Hall 4]

Direct Determination of the Actual Drug Content Incorporated into PLGA Microspheres by MALDI-TOF Mass Spectrometry

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Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been evaluated for direct determination of drug content incorporated into poly(D,L-lactic-co-glycolic acid) (PLGA) microspheres. Biodegradable PLGA (50/50) microsphere containing leuprolide acetate as

a model system was prepared by a solvent extraction/evaporation method. The leuprolide-loaded PLGA microsphere was dissolved by acetonitrile containing 0.1% TFA, and then content of leuprolide in the microsphere was directly determined by MALDI-TOF MS using alpha-cyano-4-hydroxy cinnamic acid as a matrix. Triptorelin was used as an internal standard. The relative peak height of leuprolide was calculated and plotted versus its contents. This plot showed linearity between 5 and 500 ug/mL of leuprolide and the precision was found to be in the range of 0.3 to 2.3% relative standard deviation. The results were compared to the data determined by capillary electrophoresis and HPLC. This new approach was found to be sensitive, convenient, and reliable. It is expected to be applied to various related studies including stability, peptide/protein-polymer interaction, and in vitro release study. It also provides the merits of speed, high resolution, small sample requirements, ease of determination, and simple data manipulations over other analytical tools.

[PE1-23] [04/19/2001 (Thr) 15:30 - 16:30 / Hall 4]

Stability study of PEGylated Salmon Calcitonin

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PEGylation of salmon calcitonin (sCT) has been studied to improve the bioavailability by increasing the stability of sCT. PEGylated sCT was obtained by conjugation with succinimidyl carbonate-polyethylene glycol (sc-mPEG, m.w. 5,000). Mono- and di-PEGylated sCTs were separated by GFC and showed molecular ion peaks at m/z 8339 and m/z 13274 by MALDI-TOF/MS. Mono-PEGylated sCTs were further separated into three positional isomers (M1-M3) by RP-HPLC. By LC-MS/MS analysis of tryptic digests, PEGylated sites of three isomers were identified. That is, the position of PEGylation in M1, M2 and M3 was Cys1, Lys18 and Lys11 of sCT, respectively. HPLC-UV analysis of the degradation of mono-PEGylated sCTs and sCT showed that mono-PEGylated sCTs were chemically more stable than sCT and M1 was the most stable among three mono-PEGylated sCTs. The metabolic stability study of sCT and N-terminus modified mono-PEGylated sCT using purified lysosomal enzymes, cathepsin B1 and D indicated that mono-PEGylated sCT, M1 was more stable than sCT against purified lysosomal enzymes.

[PE1-24] [04/19/2001 (Thr) 15:30 - 16:30 / Hall 4]

Preparation and Evaluation of Prostaglandin E1 (PGE1) Intraurethral Solutions for Erectile Dysfunction

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Preparation and Evaluation of Prostaglandin E1 (PGE1) Intraurethral Solutions for Erectile Dysfunction. Byoung-Ju Park*, Seung-Ho Lee, Qi-Zhe Quan, Han-Gon Choi, Jong Dal Rhee and Chul Soon Yong.

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Purpose. To prepare and evaluate PGE1 intraurethral solutions. Methods. PGE1 intraurethral solutions were prepared with polyethyleneglycol 400, propylene glycol monolaurate, and benzyl alcohol. The stability of PGE1 in intraurethral solution was investigated using a validated HPLC technique. In pentobarbital anesthetized wild cats, increases in intracavernous pressure(ICP), penile length and duration of erectile response were determined after application of PGE1 intraurethral solution. For the possible urethral mucous irritation, histological examinations were also performed. Results. It was found that PGE1 intraurethral solution was stable over 1 yr at 4℃. ICP of intraurethral solution(83.7± 15.2mmHg) was less than that of intracavernosal injection 1g of PGE1(102.5±17.7mmHg) as control.