To improve the stability of recombinant human epidermal growth factor (rhEGF) as therapeutic agent, the N-terminal PEGylated rhEGF (N-PEG-rhEGF) was prepared by site-specific bioconjugation and the stability was investigated in rat skin wound homogenates. Two different N-PEG-rhGEFs (N-PEG5K- and N-PEG20K-rhEGF) were successfully prepared with the yields of above 70%. The PEGylation site was directly confirmed by determining the molecular mass of Lys-C digested samples using MALDI-TOF MS. The biological activities of N-PEG5K-rhEGF and N-PEG20K-rhEGF were preserved 58.6% and 68.2%, respectively, compared to native rhEGF. The N-PEG-rhEGFs showed an improved stability over native rhEGF in rat skin wound homogenates. Of two N-PEG-rhEGFs, high molecular weight N-PEG20K-rhEGF was more stable. The degradation half-lives of native rhEGF, N-PEG5K-rhEGF, and N-PEG20K-rhEGFs were determined to be 0.95, 3.43, and 17.28 hours, respectively. This study indicates that PEGylation of rhEGF may increase the biological stability and has a greater potential for therapeutic use.

[PE1-21] [10/18/2002 (Fri) 13:30 - 16:30 / Hall C]

A Comparative study for single-shot immunization of diphtheria toxoid with combined PLGA microspheres.

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Biodegradable PLGA microspheres(MS) have been widely studied for delivering antigens because PLGA has the characteristics of various degradation rate. In general, since MS have shown potential for single-dose vaccines, the degradation rate of PLGA is determined by their molecular weight, polymer composition, etc. We studied the influences of molecular weight of PLGA, polymer composition and surfactant on in vitro release and in vivo effects. And a single-shot immunization of diphtheria toxoid (DT) with different MS preparation was compared to a conventional adjuvant preparation with alum. Various MS were prepared by W/O/W emulsification and solvent extraction method. MS were evaluated by particle size, surface morphology, loading efficiency, cloud point, bulk density, release test and immunization study. As decreasing the molecular weight of PLGA and using hydrophilic polymer, degradation rate increases. Amount of released antigen from low molecular weight PLGA MS and stearic acid ?added MS showed pulsatile release profile. The release of DT from surfactant-added MS was greater than that of were more than control. On the other hand, in vivo immune response by a single-shot immunization of DT with combined microsphere was equivalent to or even greater than that of three consecutive doses of conventional alum-adjuvant formulation. As a conclusion, combinations of different a good candidate for PLGA MS may be the development of effective single-shot vaccine delivery system.

[PE1-22] [10/18/2002 (Fri) 13:30 - 16:30 / Hall C]

Transfersomes-mediated gene transfer into organs in mice by direct application on intact skin

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Transfersomes are highly deformable hydrophilic lipid vesicles that are able to penetrate the skin barrier so that they can be used to carry low- and high-molecular weight molecules into the body. Until recently, it has been reported that molecules such as insulin, interleukin-2 and several other large molecules have been transported into the body using Transfersomes as a delivery system. Here however, for the very first time, genes (GFP) have been transported into the mice non-invasively using the Transfersomes as a delivery vehicle.
Transfersomes are easy to manufacture, non-toxic, stable at least for a few weeks and can also transport genes therefore Transfersomes may be further developed in the future as a non-invasive gene delivery system and can be applied in gene therapy

[PE1-23] [10/18/2002 (Fri) 13:30 - 16:30 / Hall C]

Growth inhibition of human pancreatic cancer cells by CR2945-targeted liposome

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Among the promising cancer therapy is targeting of the drug to tumor cells via receptor specific ligands. CR2945, β-[2-([2-(8-azaspiro[4.5] dec-8-ylcarbonyl)-4,6-dimethylphenyl]amino-2-oxoethyl]-(R)-1-naphthalenepropanoic acid, is known to have an inhibitory effect on a gastrin receptor of colorectal cancer cells. As the human pancreatic cancer cells (BxPC-3) express gastrin receptors, interruption of binding of gastrin with gastrin receptor of human pancreatic cancer cells by CR2945 inhibits the growth of human pancreatic cancer cells. The purpose of this study is to synergistically inhibit the growth of pancreatic cancer cells by CR2945-conjugated liposome encapsulating anticancer DNA. Conjugation of CR2945 with phospholipid(DSPE) was performed by the reaction of a carboxyl group in CR2945 with an amine group introduced into DSPE. The structural analysis of DSPE-CR2945 was carried out using FT-IR, 1H-NMR, and UV spectroscopy. The IR spectra of DSPE-CR2945 with peptide bond exhibit the characteristic bond of primary amine group at 3223cm^{-1}. The 1H-NMR spectrum of the same modified polymers shows peak at δ=8.830 which can be assigned to protons of the peptide bond. Naphthalene group of DSPE-CR2945 appears at δ=7.437~δ=7.297. The results of IR spectra and 1H-NMR spectrum show that a carboxyl group in CR2945 conjugated to an amine group in DSPE. CR2945 seems to target human pancreatic cancer cells and results from in vitro growth inhibitory study will also be presented.

[PE1-24] [10/18/2002 (Fri) 13:30 - 16:30 / Hall C]

Characterization of the rhGH released from rhGH-loaded PLGA microspheres

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The in vitro release of rhGH from PLGA microspheres was characterized. rhGH-loaded PLGA microspheres were prepared with 50:50 poly(D,L-lactide-co-glycolide) (PLGA) polymers using a double emulsion process. To simulate rhGH release under physiological conditions, the microspheres were suspended in a physiological buffer at 37℃. Quantification of the rhGH released and its molecular form analysis were carried out using SE-HPLC. Approximately 15% of the encapsulated rhGH was released within the first day, with a continuous release occurring during the following days. 95.1% of rhGH released during the first day was in the monomeric form. The monomer ratios at day 5 and day 8 were 99.4% and 98.6% respectively. At day 11 and day 14, rhGH was observed exclusively in the monomeric form. And rhGH released from microspheres was verified to be essentially in the biologically active form.
The results suggest that dimers and aggregates formed during the manufacturing process were located mostly at the surface of the microspheres and released during the early stage of release. In contrast, the rhGH in the interior of the microspheres is hypothesized to be mainly in the monomeric form, resulting in an increased monomer ratio during the mid- and late phase release.

[PE1-25] [10/18/2002 (Fri) 13:30 - 16:30 / Hall C]

Effects of Sustained-Release Formulation of Recombinant Human Growth Hormone on Body weight, Bone growth and Organs in Hypophysectomized Rats

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The rhGH-loaded PLGA microsphere formulation was prepared using a double emulsion process from hydrophilic 50:50 poly(D,L-lactide-co-glycolide) (PLGA) polymers. To investigate the sustained efficacy of this formulation, its pharmacodynamic characteristics were analyzed. It showed particle size of ca 53.1μm with high drug incorporation efficiency and it was subcutaneously administrated to hypophysectomized rats and whole body growth responses of this formulation were compared to those of the different dosing patterns of rhGH. Statistically significant increases were noted in body weight, growth plate(bone growth) and thymus size without affecting the size of other organs after 7 days at which formation of antibodies to rhGH was observed. These studies suggested that rhGH delivered continuously via these formulations showed the same efficacy on increasing body weight and bone growth as rhGH delivered via twice daily injection or osmotic minipump in