

performed in 10 mM phosphate buffer saline, pH 7.4 (containing 0.02% Tween 80), and the release properties were compared with microspheres containing native peptides. The PLGA microspheres containing native peptides demonstrated an initial rapid release in the early stage of incubation, whereas the microspheres containing PEGylated peptides rarely showed initial burst release and, instead, the release began at day 15. The release profile of PEGylated peptides from the microsphere exhibited a near zero order kinetic behavior during 20 days. Consequently, the PEG conjugation of peptide was demonstrated to be the potential way to add desirable function, (1) to minimize initial burst release and (2) to control the release rate of drug from microspheres, to the microsphere for controlled drug delivery.

[PE1-29] [10/19/2000 (Thr) 15:00 – 16:00 / [Hall B]]

Polyethylene glycol (PEG)-modified Glucagon-like Peptide-1 (GLP-1) which have increased metabolic stability and improved biological activity

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Glucagon-like Peptide-1 (GLP-1) has great potential in diabetes therapy due to its glucose-dependent stimulation of insulin secretion, but this has been limited due to its rapid degradation, primarily by dipeptidyl peptidase IV (DPP IV). To overcome this problem, GLP-1 was modified with polyethylene glycol (PEG) and the metabolic stability and biological activity was tested in vitro and in vivo. Mono-PEGylated GLP-1 was separated by size-exclusion chromatography (SEC) and characterized by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry. Incubation of GLP-1 with DPP IV resulted in degradation of native GLP-1 (7-36) amide to GLP-1 (9-36) amide, while mono-PEG-GLP-1 was resistant to DPP IV degradation. The in vitro and in vivo biological activities were investigated by measuring cyclic AMP production in RINm5F cells and blood glucose levels lowered after intravenous administration to rats, respectively. The results of the experiments demonstrate that modification of GLP-1 with PEG increased the plasma stability against DPP IV without impairing significantly its insulinotropic activity. This may indicate that this modification can improve the potential of GLP-1 in the treatment of type-II diabetes.

[PE1-30] [10/19/2000 (Thr) 15:00 – 16:00 / [Hall B]]

Characterization of Carbohydrate-Directed PEGylation of Ricin A Chain

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Ricin A chain, toxic subunit of plant toxin ricin from *Ricinus Communis*, has been modified by carbohydrate-directed PEGylation to prolong circulating half-life. The site-directed PEGylation of the carbohydrate was compared to non-specific PEGylation of amino residues of RTA. The carbohydrate of RTA was oxidized with sodium m-periodate and modified with monomethoxy polyethylene glycol-hydrazide (MPEG-HZ, MW 5,000). PEGylation of amino group of RTA was performed with mPEG-succinimydyl propionate (MPEG-SPA, MW 5,000). The PEG-modified RTAs were purified by size-exclusion HPLC and characterized by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). RTA, RTA-MPEG-HZ and RTA-MPEG-SPA were radioiodinated by chloramine T-method and injected i.v. to rats. Blood clearance and tissue distribution were investigated. The systemic clearance of the RTA-MPEG-HZ and RTA-MPEG-SPA were reduced 64.8% and 34.1%, respectively, compared to native RTA. It was observed that RTA-MPEG-HZ was accumulated in the liver less than the RTA-MPEG-SPA and native RTA. The liver uptake of the RTA-MPEG-HZ and RTA-MPEG-SPA were reduced 62.4% and 32.6%, respectively, compared to native RTA. The kidney uptake of both RTA-MPEG-HZ and RTA-MPEG-SPA was also lower than that of native RTA. The site-directed PEGylation of the

carbohydrate of RTA significantly decreased uptake by liver and kidney, and resulted in prolonged circulating half-life. These results suggest that RTA prepared by carbohydrate-directed PEGylation would be more effective when constructed as immunotoxin for tumor targeting.

[PE1-31] [10/19/2000 (Thr) 15:00 – 16:00 / [Hall B]]

Comparative re-evaluation of protein denaturation for PLG microspheres prepared by W/O/W multiple emulsification processes.

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The purpose of this study was to re-evaluate the degree of protein denaturation during microencapsulation with poly (lactide-co-glycolide) (PLG) polymer. Ovalbumin(OVA), a model antigen, was entrapped in PLG microspheres by W/O/W multiple emulsification method in various conditions, i.e. with or without addition of polyvinylpyrrolidone(PVP) as a stabilizer for primary emulsification. PLG microspheres were suspended in PBS solution(pH 7.4) and incubated with shaking at 37.5°C. Release medium was collected periodically and analyzed using the micro-BCA protein assay method and ELISA method. In preliminary study with SDS-PAGE, typical bands for OVA showed that the primary structure of protein was not affected significantly. However, there was a decrease in immunoreactivity of OVA. In order to express the degree of protein denaturation, antigenicity ratio(AR) was introduced as follows: amount of immunoreactive of OVA / total amount of OVA released × 100(%). Especially the addition of primary emulsification stabilizer greatly influenced on protein denaturation : i.e. about 65% of AR with stabilizer versus less than 35% of AR without stabilizer. Therefore, in order to obtain information on structural integrity of protein, it is better to re-evaluate the protein denaturation employing immunoreactivity measurement for antigen entrapped in PLG microspheres.

[PE1-32] [10/19/2000 (Thr) 15:00 – 16:00 / [Hall B]]

Intranasal Delivery of Poly(ethylene glycol) Conjugated Salmon Calcitonin in Rats

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Activated PEG molecules (2000 and 5000 Da) were attached to salmon calcitonin via covalent linkage. Mono-PEG-sCT was then separated by size exclusion chromatography and characterized by CE and MALDI-TOF mass spectrometry. Six Male Sprague-Dawley rats (220-280 grams) per group were used. sCT (2 IU/kg) and PEG-sCT (2 IU/kg) were dissolved in saline solution and directly applied onto the nasal mucous membrane of rat by using micropipet (50 ul/rat) under anesthesia, respectively. Blood was withdrawn at a certain time intervals and centrifuged to collect the plasma samples. Serum calcium concentration was measured by o-cresolphthalein complexone complex assay using UV spectrophotometer. Serum calcium concentrations following nasal placebo administration remained around 100 % of the basal calcium levels during 6 hours. Nasal administration of native sCT (2 IU/kg) resulted in a slight decrease in serum calcium with a maximum decrease of less than 10 % at 30 min and returned to the control level within two hours. Significantly prolonged decrease of serum calcium were observed over six hours after nasal administration of PEG-sCTs. It was also observed that PEG5000-sCT had the less hypocalcemic effect than PEG2000-sCT. Therefore, these results indicate that PEG attachment to sCT can be investigated as a novel nasal delivery system of therapeutic peptides.

[PE1-33] [10/19/2000 (Thr) 15:00 – 16:00 / [Hall B]]

Effect of Enhancers on the in vitro Skin Permeation of Ciclopirox from novel Soft