

Podia Schedule

Lindquist room

8:30-8:50 AM	PD 1	Bhalla, Shaifali	University of Illinois <i>ENDOTHELIN-A RECEPTOR BLOCKADE IN THE CNS ENHANCES MORPHINE ANALGESIA AND ELIMINATES MORPHINE TOLERANCE IN RATS</i>
8:55-9:15 AM	PD 2	Rai, Aarati	University of Illinois <i>ENDOTHELINB RECEPTOR STIMULATION ENHANCES THE DELIVERY OF PACLITAXEL SELECTIVELY TO THE BREAST TUMOR TISSUE</i>
9:20-9:40 AM	PD 3	Ganguly, Manjori	University of Nebraska Medical Center <i>STRUCTURE OF A DNA DUPLEX CONTAINING A MODIFIED NUCLEOTIDE</i>
9:45-10:05 AM	PD 4	Khor, Hui Koon	University of Kansas <i>THE OXIDATIVE INACTIVATION OF GROEL: CYS/MET?</i>
10:10-10:20 AM	PD 5	Sriadibhatla, Srikanth	University of Nebraska Medical Center <i>EFFECT OF PLURONIC BLOCK COPOLYMERS ON GENE EXPRESSION</i>
10:50-11:10 AM	PD 6	Shikaya, Ronald	University of Nebraska Medical Center <i>INTRAMOLECULAR DNA TRIPLEXES: ENERGETIC AND ELECTROSTATIC CONTRIBUTIONS</i>
10:25-10:45 AM	PD 7	Likitlersuang, Sukhon	University of Iowa <i>THE INFLUENCE OF MAGNESIUM STEARATE ON THE COMPACT DEFORMATION MECHANISM</i>
11:15-11:35	PD 8	Koo, Otilia	University of Illinois <i>DELIVERY OF CAMPTOTHECIN IN NANOSIZED PEGYLATED PHOSPHOLIPID MICELLES</i>

Break for lunch

Podia Schedule

Lindquist room

1:30-1:50	PD 9	Oh, Kyung	University of Nebraska Medical Center <i>PREPARATION, PROPERTIES AND REACTIONS OF POLYELECTROLYTE NETWORKS WITH OPPOSITELY CHARGED MOLECULES</i>
1:55-2:15	PD 10	Guo, Chunqiang	University of Illinois <i>ASSESSMENT OF A TERNARY TRANSFECTION SYSTEM</i>
2:20-2:40	PD 11	Rytting, Erik	University of Kansas <i>CHARACTERIZATION AND INHIBITION OF PLACENTAL OCTN2-MEDIATED CARNITINE UPTAKE IN BEWO CELLS</i>
2:45-3:05	PD 12	Tauro, Jovita	University of Illinois <i>EVALUATION OF A MMP TARGETED PEPTIDE FOR ACTIVATION OF CISPLATIN</i>
3:10-3:30	PD 13	Vasir, Jaspreet	University of Nebraska Medical Center <i>ENTRAPMENT OF BIOLOGICALLY ACTIVE AND STRUCTURALLY UNPERTURBED LYSOZYME IN PLGA MICROPARTICLES</i>
3:40-4:00	PD 14	Yan, Xiaoliang	University of Illinois <i>PHYSICOCHEMICAL PROPERTIES AND ACUTE IN VIVO TOXICITY OF POLY-(ACRYLIC ACID-CO-METHYL METHACRYLATE) MICROPARTICLES</i>
4:05-4:25	PD 15	Lu, Enxian	University of Illinois <i>STREPTOMYCIN LOADED CHITOSAN NANOPARTICLES: OPTIMAZIATION, CHARACTERIZATION AND ORAL EFFICACY STUDY</i>

Podia Abstracts



ENDOTHELIN-A RECEPTOR BLOCKADE IN THE CNS ENHANCES MORPHINE ANALGESIA AND ELIMINATES MORPHINE TOLERANCE IN RATS

S. Bhalla, G.A. Matwyshyn, and A. Gulati

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Purpose. We have shown that endothelin (ET) mechanisms in the CNS are involved in analgesic actions of morphine. The present study was conducted to evaluate *in vivo* and *in vitro* effects of ET_A receptor antagonists, BQ123 and BMS182874, on morphine tolerance. **Methods.** Tolerance to morphine was induced in male rats by implantation of 6 morphine pellets (75 mg/pellet, s.c.) over a 7-day period. Analgesic response was measured by tail-flick latency test. Radioactive ligand binding assays were conducted to determine the effect of ET_A receptor antagonists on opioid receptor binding and stimulation of G-proteins in the brain of control and morphine tolerant animals. **Results.** In morphine tolerant rats, tail-flick latency was significantly lower ($P < 0.05$) compared to placebo group. BQ123 and BMS182874 significantly increased ($P < 0.05$) analgesic response in placebo group by 30.0% and 30.2%, respectively. In morphine tolerant rats, analgesic response was significantly potentiated ($P < 0.05$) by both BQ123 (94.5%) and BMS182874 (66.7%). BQ123 potentiated analgesia was blocked by opioid receptor antagonist, naloxone, indicating an opiate-mediated effect. However, [³H]naloxone binding to opioid receptors in the brain was not affected by BQ123 or BMS182874, suggesting that ET_A antagonists did not bind directly to opiate receptors. Morphine and ET-1 induced GTP stimulation was significantly lower ($P < 0.05$) in morphine tolerant group (33% and 42%, respectively) compared to control group. BQ123 and BMS182874 significantly increased ($P < 0.05$) G-protein activation in morphine tolerant group (96% and 86%, respectively) compared to control group. **Conclusion.** These results suggest that uncoupling of G-protein occurs in morphine tolerance. ET_A receptor antagonists restore coupling of G-protein to its receptors, thereby restoring analgesic effect. These findings indicate that a combination of ET_A receptor antagonists and opiate analgesics could provide a novel approach in improving analgesia and eliminating tolerance.

ENDOTHELIN_B RECEPTOR STIMULATION ENHANCES THE DELIVERY OF PACLITAXEL SELECTIVELY TO THE BREAST TUMOR TISSUE

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Purpose: Tumors blood vessels grow differently from normal tissue vasculature due to changes in the production of growth factors and vasoactive substances. We have found that administration of endothelin-1 (ET-1) to breast tumor bearing rats increased blood flow selectively to the tumor tissue by stimulating ET_B receptors. The purpose of the present study is to determine whether anticancer drug, paclitaxel, delivery to tumor tissue can be enhanced using ET_B receptor agonist, IRL1620. **Methods:** Breast tumors were induced in female Sprague Dawley rats by N-methyl-n-nitrosourea (MNU, 50 mg/kg, i.p). Tumor bearing rats were treated with either saline (0.3 ml/kg, i.v.) or IRL1620 (5.5 μg/kg, i.v.). [³H]-paclitaxel (40 μCi, i.v) was administered 15 minutes after saline or IRL1620 administration. Perfusion to the tumor was measured using laser doppler technique. Plasma, liver, lung, kidney, spleen and tumor tissues were collected 3 hours after [³H]-paclitaxel administration to determine its concentration. Pharmacokinetic studies were conducted in similar groups treated with paclitaxel. Serial plasma samples were collected at baseline, 0.5, 2, 6, and 10 hours for HPLC analysis. **Results:** IRL1620 produced a significant increase (150%, $p < 0.005$) in tumor perfusion, which lasted for 90 minutes. IRL1620 did not produce any change in [³H]-paclitaxel concentration in the plasma, lungs, kidney, and liver compared to saline treated rats. However, a marked increase in [³H]-paclitaxel concentration in the tumor tissue (453%, $p < 0.0001$) and spleen (43%, $p < 0.05$) was observed in IRL1620 treated rats. Plasma pharmacokinetic profile of paclitaxel was not affected by IRL1620. **Conclusions:** Results indicate that ET_B receptor agonist, IRL1620, significantly enhanced perfusion and concentration of [³H]-paclitaxel selectively in the breast tumor. Therefore, ET_B receptor agonists can be used to enhance the delivery of paclitaxel to the breast tumor tissue.

STRUCTURE OF A DNA DUPLEX CONTAINING A MODIFIED NUCLEOTIDE

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Purpose: Sequence dependent electrostatic landscape of DNA contributes, in part, to site-specific recognition of the double helix by small ligands and proteins. To elucidate the effect of charge localization on DNA structure we have used a model that does not require protein molecules. In this model amino acid like sidechains are directly attached onto an oligonucleotide in order to mimic the action of protein sidechains in the major groove of DNA. The results will facilitate rational design of new DNA binding drug molecules.

Methods: We synthesized 5-(3-aminopropyl)-2'-deoxyuridine residue (Z3dU) and this was incorporated into oligomers using standard phosphoramidite chemistry. A combination of molecular biology techniques like DNA foot-printing and gel shift assay along with spectroscopy, optical and calorimetric melting techniques were used to study the orientation of the modified amino acid like sidechain and its effect on the structure of DNA.

Results: Foot-printing studies on the modified DNA indicated that the modified sidechain oriented towards the the 3' direction. NMR studies of the modified DNA resulted in a 0.24 ppm upfield shift of one ³¹P resonance. The perturbed ³¹P resonance was at the phosphodiester linkage toward the 3'-side of the modified residue which indicates that the Z3dU amino group did not participate in a salt bridge with its 5'-phosphate. Thermodynamic studies showed that the incorporation of modified residues can either decrease (~4°C) or increase (~4°C) the T_M of the host oligomer depending on the sequence. Studies from gel mobility shift assay showed that incorporation of modified residues resulted in reduced mobility of the duplex and the reduction increased with increasing number of modified residues.

Conclusion: The incorporation of modified residues lowers the stability of duplex DNA and the presence of one charge can have significant effect on the structure of DNA however the effect seems to be sequence specific.

THE OXIDATIVE INACTIVATION OF GROEL: CYS/MET?

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Purpose: GroEL has been previously shown to be inactivated by the reactive oxygen species (ROS) hypochlorous acid (HOCl) and peroxynitrite (ONOO⁻) with concomitant loss of Cys content and formation of Met sulfoxide. The next step was to determine which of the amino acid modifications was the cause of the inactivation.

Methods: GroEL (17.5 μM) was treated with ROS in pH 7.5 phosphate buffer, and the reactions terminated by adding Met. Activity was determined through refolding of a denatured malate dehydrogenase. Reversibility of inactivation was determined by incubating ROS treated GroEL with either dithiothreitol (DTT) alone or methionine sulfoxide reductase (MsrB/A) and DTT. Thioglobulin was used to quantify free thiols (Cys). Size exclusion chromatography was used to determine changes in global conformation. Oxidation products were characterized by tryptic digestion and LC/MS/MS analysis.

Results: Inactivation of GroEL correlates with the conversion of oligomeric into monomeric GroEL suggesting that the inactive form of HOCl-treated GroEL is monomeric. This is in contrast to ONOO⁻, where 0.25mM oxidant does not cause any significant change in. cause any significant change in oligomeric structure but ca. 30% of the activity is lost. Incubation with DTT did not recover activity of GroEL for either ROS, implying that reversible oxidation products such as disulfides, or Cys sulfenic acid are not involved. The MsrB/A system was able to recover partial activity for 0.05-0.25mM HOCl.

Conclusion:

Met sulfoxide formation is part of the cause for inactivation in HOCl-treated GroEL. The activity not recovered by the MsrB/A system is likely due to Met sulfone formation. Oxidation by HOCl and ONOO⁻ may be different due to the nature of Cys oxidation products (Cys sulfonic acid detected for HOCl but not ONOO⁻) and/or a differential selectivity for specific Met residues (ONOO⁻ does not react with Met¹¹¹ or with Met¹¹⁴), which also results in a differential in loss of oligomeric structure.

Effect of Pluronic Block Copolymers on Gene Expression

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University of Nebraska Medical Center

Purpose: To study the effect of Pluronic block copolymers (PBC) on gene expression. **Methods:** NIH3T3, CHO and Cl66 cells were stably transfected with reporter genes expressing luciferase and green fluorescent protein (GFP) by using routine molecular biology techniques. Various pluronics were incubated with these cells and luciferase expression was measured by the luminescence and GFP with the aid of flow cytometry. **Results:** Pluronic P85 demonstrated significant increase in the luciferase activity in Luc-NIH3T3 cells treated with the block copolymer. Control experiments using the same concentrations of PBC demonstrated that it does not affect the luciferase - luciferin reaction. Furthermore, no effect on the luciferase activity was observed when the cells were exposed to the PBC (3hrs) and lysed immediately without further incubation. This suggests that PBC induces luciferase gene expression in the cells during 24hr period of the incubation. This was confirmed in other cell models and also by measuring the mRNA levels using real time RTPCR. Of the PBC tested molecules with intermediate hydrophilic-lipophilic balance (HLB 9-16) and relatively large hydrophobic block (39-69 PO units), such as P123, P103, L64 and P85 were found to be the most effective (at ca 0.3% w/v). The hydrophilic block copolymers, F127 and F88 (HLB 22 and 28) and block copolymers with relatively short PO block, L35 and L61 (16 and 31 PO units) were much less active. Furthermore, the polycation-based transfection system that contained a mixture of free P123 and its conjugate with polyethyleneimine (PEI) was shown to significantly induce the expression of the reporter genes in these cell models. **Conclusions:** PBC alone and in combination with polycation can alter gene expression through the mechanisms that differ from the delivery of the DNA into the cell and may induce expression of genes that are already present in the cells.

INTRAMOLECULAR DNA Triplexes: ENERGETIC AND ELECTROSTATIC CONTRIBUTIONS

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Triple helical structures form when a third strand binds to the major groove of a nucleic acid duplex through Hoogsteen base pairing. Triplexes are a subject of great interest due to their ability to interfere with gene expression, allowing new therapeutic applications, especially as antigenic therapy in cancer treatment. The inhibition of gene expression involves the formation of stable triplexes under physiological conditions. It is important, then, to further our understanding of the energetic and electrostatic contributions controlling the stability and melting behavior of DNA triplexes.

We have used a combination of temperature-dependent UV spectroscopy and differential scanning calorimetric (DSC) techniques to investigate the unfolding of an intramolecular triplex, d(A₇C₅T₇C₅T₇), and related triplexes containing single TTA → TAT substitutions in its stem of 7 TAT base triplets. Specifically, we obtained standard thermodynamic profiles (T_M , ΔG° , ΔH , and $T\Delta S$) for the unfolding of four triplexes as a function of salt concentration. These triplexes unfold in monophasic or biphasic transitions (triplex→duplex→coil) depending on the concentration of salt and position of the substitution. However, their T_M 's were independent of strand concentration, confirming their intramolecular folding. The incorporation of a single TTA → TAT substitution destabilizes triplex formation (association of the third strand) and this destabilization is larger at low salt concentrations. The magnitude of these destabilizations is due to less favorable enthalpy contributions, which strongly depends on the position of the substitution.

The Influence of Magnesium Stearate on the Compact Deformation Mechanism

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Purpose. To study the effect of binary compact compositions on Hiestand bonding indices (BI) of binary maltodextrin compacts with various magnesium stearate concentration levels.

Methods. Three commercial grades of Maltrin[®], M040, M100 and M150 were used to prepare binary powder mixtures (M040-M150 and M040-M100) to represent materials with a wide range of brittleness. For each mixture, magnesium stearate was added at concentrations of 0%, 0.16%, 0.32%, 0.48%, and 0.64%. Compacts of each lubricated mixture were prepared for BI determination. Statistical analysis using SAS software was conducted to develop models for predicting the relationship between binary compositions and BI under various concentrations of magnesium stearate.

Results. Maltodextrin M040, M100 and M150 have different deformation mechanisms under compression. M150 is substantially more brittle than M100 and M040. Binary mixtures of these materials provide a wide range of deformation mechanism for study. Without magnesium stearate, a linear relationship was observed between BI and the composition of the binary systems of M040-M150 and M040-M100 (P value < 0.01). With magnesium stearate at a concentration ranging from 0.16% to 0.64%, second-degree polynomial relationships between BI and binary mixture compositions were observed (P value < 0.01 for both binary systems). Moreover, at 0% to 0.16% magnesium stearate, BI of the M040-M100 was higher than that of M040-M150 systems. As magnesium stearate concentration was higher than 0.32%, BI of M040-M100 was lower than that of M040-M150.

Conclusions. The BI appears to be useful for the prediction of the influence of magnesium stearate on powder compacts with different deformation mechanisms. Hence BI is useful for studying the sensitivity of pharmaceutical excipients to magnesium stearate.

Acknowledgment. The financial support provided by Abbott Laboratories is gratefully acknowledged.

DELIVERY OF CAMPTOTHECIN IN NANOSIZED PEGYLATED PHOSPHOLIPID MICELLES

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Purpose: The aim of this study was to improve camptothecin (CPT) solubilization in PEGylated phospholipid micelles. Two methods of formulation, a novel pH change method and the film coprecipitation/reconstitution method, will be compared. The former is based on CPT reversible, chemical conversion from the relatively water-soluble carboxylate to water-insoluble active lactone form for micellar loading at lower pH. Lyophilization of CPT-PEGylated phospholipid micelles (CPT-SSM) will also be conducted. **Methods:** CPT carboxylate was added to DSPE-PEG2000 micelles (5 mM) using various CPT:DSPE-PEG2000 molar ratios (0.009:1 to 0.086:1) and maintained at pH 5 and 25°C for up to 2 days for complete CPT carboxylate-lactone conversion and equilibrium lactone solubilization as CPT-SSM. For comparison, similar CPT:DSPE-PEG2000 molar ratios were used to prepare CPT-SSM by coprecipitation/reconstitution as described previously¹. Lyophilization of CPT-SSM was conducted using the lyophilization cycle described previously². Excess CPT was removed by centrifugation. CPT-SSM was characterized by TEM, quasi-elastic light scattering, RP-HPLC and fluorescence for morphology, mean diameter, solubilized CPT concentration and spectroscopic properties, respectively. **Results:** CPT-SSM prepared by both methods were ~14 nm, solubilizing 25-fold higher CPT and CPT fluorescence peak emission wavelengths (430 nm) were not significantly different, implying that CPT was loaded into the same region of the SSM. There was no significant change in CPT-SSM properties after lyophilization and reconstitution (p>0.05). **Conclusions:** CPT-SSM was successfully prepared using 2 formulation methods that can be used to load CPT reproducibly into lipid-based carriers. CPT-SSM can be successfully lyophilized without the use of lyo- and cryo-protectants. ¹ O. Koo, et al, *Proc. 29th Annual Meeting Control. Release Society, 698-699, 2002.* ² O. Koo, et al, *Proc. 31st Annual Meeting Control. Release Society, in press, 2004*

Preparation, Properties and reactions of Polyelectrolyte Networks with oppositely charged molecules,

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Purpose To examine the physicochemical properties of the cross-linked polyelectrolyte-poly(ethylene oxide) (PEO) hydrogels and the reactions of these gels with oppositely charged compounds and to develop the polymeric hydrogel systems for the delivery of biomacromolecules such as proteins and hormones.

Methods A cross-linked poly(acrylic acid) (PAA) and composite PAA-cl-PEO hydrogels were synthesized by free-radical polymerization using the redox initiator system. The concentration of carboxyl groups was determined by potentiometric titration. UV/Vis spectrometry was used to characterize the reaction of hydrogels with surfactant, polycations, and protein.

Results The hydrogels with various cross-linking densities were synthesized. The gels prepared had *ca.* 0.033–0.041 mmol –COOH groups per 1g of swollen gels and exhibited relatively high swelling capacity. The swelling capacity of gels depended on environmental factors such as pH, concentrations and chemical nature of salts. The reaction of these networks with a cationic surfactant, cetylpyridinium bromide, and polycations (poly(N-alkyl-4-vinylpyridinium) salts and their PEO block copolymers) resulted in the neutralization of polyion segments in the network, followed by the collapse of gels. Furthermore, sorption of protein, cytochrome C, by polyelectrolyte networks resulted in the formation of polyelectrolyte-protein complex. By adding Ca^{2+} , the absorbed proteins can be released from the complex.

Conclusions. PAA and (PAA)-cl-(PEO) hydrogels exhibited the pH and salt induced swelling response, and formed the polyelectrolyte complexes by reacting with surfactant, polycations, and protein. The hydrogels can be favorable candidates for tissue engineering and drug delivery systems.

ASSESSMENT OF A TERNARY TRANSFECTION SYSTEM

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Purpose: To study the mechanism of enhancing effect of plasmid-dendrimer-silica system on transfection efficiency.

Methods: Alexa Fluor[®] 647 labeled LacZ plasmid DNA was incubated with SuperFect[®] transfection agent (partially degraded dendrimer) for 5-10 min. Alexa Fluor[®] 488 labeled silica particles were then added into the mixture to form a ternary system. This transfection system was incubated with CHO-k1 cells on a 24-well plate at a density of 6.26×10^4 cells/well for 3 h. Untreated cells and plasmid DNA treated cells were controls. Cells were rinsed with DPBS and incubated with fresh medium for 24 h. Cells were then harvested and suspended in serum-free media and examined using FACS Vantage Flow Cytometry. Ten thousand cells were counted and three independent runs of each sample were reviewed. Confocal microscopy was also carried out to detect the uptake of particles into the cell.

Results: The increase of production of beta-galactosidase was observed in plasmid-dendrimer-silica treated groups compared to DNA control group. Three ternary system treated groups had significantly higher level of beta-galactosidase expression compared to DNA-dendrimer group. Flow cytometry results demonstrated that both DNA- and silica- positive cells and DNA-silica double positive cells were present in the ternary system treated samples. However the percentage of total DNA-positive cells was not significantly different between the DNA-dendrimer cells and DNA-dendrimer-silica treated cells. Results from confocal microscope suggested these ternary transfection complexes were taken up by cells.

Conclusions: Plasmid-dendrimer-silica ternary system enhances transfection efficiency based on protein production. These ternary transfection complexes can be taken up by the cell. Silica particles were suggested to act as a second transfection agent by increasing the production of protein in each cell.

CHARACTERIZATION AND INHIBITION OF PLACENTAL OCTN2-MEDIATED CARNITINE UPTAKE IN BEWO CELLS

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Purpose: The placental transport of carnitine is significant because the fetus cannot supply itself with adequate amounts of this nutrient. Carnitine deficiencies in infants can lead to symptoms ranging from muscle weakness to sudden infant death. Objectives of this study include the characterization of OCTN2 function in the BeWo cell line and the inhibition of placental carnitine uptake by amphetamine derivatives.

Methods: BeWo cells, an *in vitro* model of human placental trophoblasts, were seeded in 12- or 24-well tissue culture plates and incubated at 37°C until monolayers were confluent (4-6 days). Uptake studies with radiolabeled L-carnitine and inhibitors in HBSS were carried out in the plates at 37°C for 30 minutes. Cells were immediately washed with cold HBSS and lysed for 2-3 hours prior to quantitation by scintillation spectrometry.

Results: Western blotting confirmed the expression of OCTN2 in BeWo cells. Uptake of L-carnitine in BeWo cells was Na^+ -dependent and saturable ($K_m = 9.8 \pm 2.4 \mu\text{M}$, $V_{max} = 800 \pm 70 \text{ pmol/mg protein/30 min}$) with a non-saturable constant of $2.8 \pm 0.3 \mu\text{L/mg protein/30 min}$. IC_{50} values for inhibition of carnitine uptake are presented for known OCTN2 inhibitors and amphetamine derivatives. A relationship between the chemical structure of the analogs and the extent of inhibition was observed. Lineweaver-Burk plots show that inhibition by TEA, valproate, and ephedrine was competitive.

Conclusions: High-affinity carnitine uptake in the BeWo cell line is mediated by OCTN2. Inhibition of carnitine transport by amphetamines potentially poses serious consequences for fetal development.

EVALUATION OF A MMP TARGETED PEPTIDE FOR ACTIVATION OF CISPLATIN

J. R. Tauro and R. A. Gemeinhart
The University of Illinois

Purpose: To evaluate a potential targeting strategy for cisplatin using a metalloprotease peptide substrate for targeted release of cisplatin from a polymeric wafer.

Methods: Peptide was incubated with matrix metalloprotease MMP-2 or MMP-9. Cleavage products were studied using reverse phase HPLC with fluorescence detection. To assess the *in vitro* effectiveness, cisplatin, cisplatin bound to the cleavage fragment, cisplatin bound to the peptide, the peptide, and the cleavage fragment were incubated for 24 hours with U-87MG cell line. Cell viability was measured using the modified MTS assay. Cells were observed under a fluorescence microscope after staining with ethidium homodimer (stains dead cells) and the fluorescently labeled peptide. Extent of conjugation of the peptide with the monomer for the wafer poly(ethylene glycol) diacrylate via Michael addition was studied at different pHs by following the reduction in the free sulfhydryl groups using Ellman's reagent.

Results: HPLC results prove that the peptide was cleaved between the expected amino acids. The peptide was cleaved (100%) within 2.5 hours by MMP-2 and 70% in 4 hours by MMP-9. Cisplatin complexed fragments of this peptide had EC_{50} of 87.66 μM which is comparable to the effectiveness of cisplatin ($\text{EC}_{50} = 37.39 \mu\text{M}$). The peptide and the fragment alone were not cytotoxic. Michael addition was confirmed by free sulfhydryl groups reduction within 24 hours, indicating that the peptide readily conjugated with the PEGDA. Optimal conjugation conditions were observed under nearly neutral conditions.

Conclusion: The selected peptide was a substrate for MMP-2 and MMP-9 although some selectivity was observed. The peptide-cisplatin conjugates exhibited limited toxicity while the cytotoxicity of cisplatin is retained following cleavage of the peptide. The peptide readily conjugates with the monomer to be used in the polymeric system. Hence, this system may be used to target tumors.

PD13

Entrapment of biologically active and structurally unperturbed lysozyme in PLGA microparticles

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University of Nebraska Medical Center

Purpose: To achieve high entrapment efficiency of biologically active and structurally unperturbed lysozyme in PLGA microparticles for controlled release formulation. **Methods:** Microparticles of poly D, L-lactide-co-glycolide (50:50 PLGA) containing lysozyme were formulated using a water-in-oil-in-water double emulsion solvent evaporation method. Formulation parameters were optimized to improve entrapment of lysozyme in microparticles. The optimized formulation parameters were used for studying the effect of different additives in the formulation to study the structure and bioactivity of encapsulated lysozyme. Each formulation was analyzed for protein loading, burst effect, and *in vitro* release of lysozyme. Lysozyme activity was determined using a turbidimetric method by monitoring the rate of lysis of bacterial cell walls of *Micrococcus lysodeikticus*. The conformation of lysozyme encapsulated in PLGA microparticles was determined using Fourier-transform infrared (FTIR) spectroscopy. **Results:** Addition of 0.5% w/v Polyvinyl alcohol (PVA) to the internal aqueous phase (IAP) prevented denaturation of protein at the w/o interface and retained (64% of) its biological activity. However, the presence of 3% w/v sodium bicarbonate in the IAP not only improved the entrapment efficiency of lysozyme by 25 folds, but also sustained its release for 26 days under *in vitro* conditions. Lysozyme retained 54% of its bioactivity when salt was added as against 21% without it. Lysozyme was present in its native secondary conformation (Amide I region) within the microparticles, except for the microparticles prepared with no additive in IAP. Peaks at 1625 and 1695 cm^{-1} in the second derivative FTIR spectra were attributed to the formation of non-covalent aggregates of lysozyme at the w/o interface. **Conclusion:** The formulation strategy of using an alkaline salt and an emulsifier in the IAP helped in attaining microencapsulation of structurally unperturbed and biologically active protein in microparticles.

PD14

PHYSICO-CHEMICAL PROPERTIES AND ACUTE *in vivo* TOXICITY OF POLY-(ACRYLIC ACID-CO-METHYL METHACRYLATE) MICROPARTICLES

Xiaoliang Yan and Richard Gemeinhart
University of Illinois

Purpose: To develop a microparticulate drug delivery system.

Methods: Poly-(acrylic acid-co-methyl methacrylate) (pAA-MMA) microparticles ($\mu\Phi$) were prepared by free radical emulsion polymerization in aqueous media. Cisplatin was loaded into pAA-MMA $\mu\Phi$ by complexation mechanism. The particle size, morphology, zeta potential, and *in vitro* cytotoxicity on NIH/3T3 cell line were characterized for both unloaded microparticles and cisplatin-loaded microparticles. In addition, titration using 0.1N NaOH aqueous solution was performed to estimate the relative carboxylic acid content in pAA-MMA $\mu\Phi$. *In vitro* release of cisplatin from pAA-MMA $\mu\Phi$ was monitored using cisplatin PBS suspensions as controls. Acute toxicity and LD_{50} of both unloaded and cisplatin-loaded microparticles on Sprague Dawley rats were evaluated by modified Up-and-Down Procedure (UDP).

Results: The pAA-MMA $\mu\Phi$ s of different monomer molar ratios were prepared. Dynamic light scattering measurement showed particle sizes ranging from 500nm up to 2 μm depending upon the monomer molar ratio chosen. Size was confirmed by scanning electron microscopy. Zeta potentials were affected by the molar ratio and cisplatin loading. *In vitro* release of cisplatin from pAA-MMA microparticles indicated a strong interaction between cisplatin and microparticles that only allowed about 60% release in 6 days. *In vitro* cytotoxicity tests proved that microparticles are non-toxic to the cell line used. Microparticles exhibited low IV toxicity which suggests that this particulate system can further be utilized for drug delivery.

Conclusion: We conclude that a non-toxic pAA-MMA $\mu\Phi$ has been synthesized. The characterization of the $\mu\Phi$ indicated that AA content in copolymer determined several key properties. *In vitro* release of cisplatin was prolonged which will allow localization of drug delivery. This microparticulate system warrants further characterization as a potential drug delivery system.

PD15

STREPTOMYCIN LOADED CHITOSAN NANOPARTICLES: OPTIMIZATION, CHARACTERIZATION AND ORAL EFFICACY STUDY

E. Lu, C. Popescu, and H. Onyuksel
University of Illinois at Chicago

Purpose: To develop streptomycin loaded chitosan nanoparticles for oral delivery

Methods: The streptomycin loaded chitosan nanoparticles were prepared using the conventional TPP method and was developed and characterized with respect to their size, zeta potential, SM incorporation efficiency and release. Furthermore, the *in vivo* efficacy of SM chitosan nanoparticles was studied using a *M. tuberculosis* (TB) chronic infection mouse model.

Results: The mean size and zeta potential values of optimal SM-chitosan nanoparticles were $557.93 \pm 100.38 \text{nm}$ and $+52.07 \pm 3.4 \text{mV}$ respectively. SM incorporation efficiency was $52.11 \pm 0.71\%$. *In vitro* release study showed that the chitosan nanoparticles can retain most of the loaded drug at pH 1.2 and pH 7.4 buffers. The *in vivo* efficacy study indicated that oral SM-chitosan nanoparticles showed a 1 \log_{10} reduction ($p < 0.01$) in growth of the bacilli and were as effective as subcutaneously injected aqueous SM solution.

Conclusions: The SM chitosan nanoparticle developed in this study demonstrate an acceptable *in vitro* characteristics and a high *in vivo* efficacy after oral administration, therefore can be a promising oral delivery product of SM.