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• Repair of Osteochondral Defects in the Rabbit With a Novel, Thrombin-Related Peptide

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The successful repair of damaged articular canilage remains one of the most difficult challenges in orthopaedic surgery. We have previously reported that TP508, a synthetic 23 amino acid peptide representing a receptor-binding domain of human thrombin, accelerates fresh fracture healing in animals. In this study, we tested the effects of TP508, formulated in controlled release microparticles, on healing of critical size articular canilage defects in rabbits. A critical size cylindrical defect (3-mm diameter, 1.5-2 mm depth) detect was made in the trochlear groove of the femur of young, male New Zealand white rabbits (2-3 kg, n=15). Bilateral defects were filled white 20/m PLGA porous controlled release microspheres containing either 10 or 50 ug TP508 peptide per defect, with the controls containing PLGA alone. Animals were sacrificed at 9 weeks. Histological sections were assessed qualitatively and quantitatively (maximum possible score is 24.) The results show the control (PLGA alone) repair tissue was characterized as mostly fibrocartilage with poor quality joint surfaces and with poor integration at the junction between repair and native tissue. TP508-treated defects exhibited a predominantly hyaline matrix with evidence of significant aggrecan content as shown by positive safranin-O staining. With good integration at the junction between repair and native tissue. Histological quantification showed that the control defects had a mean score of 9.4 ± 1.6. In comparison, treatment with 10 and 50 ug of TP508 had scores of 18.6 ± 1.4 and 19.8 ± 1.0, respectively, a statistically significant improvement (p < 0.05, ANOVA). Overall, the quality of canilage repaired with TP508 was significantly enhanced over control defects. These results provide support for further development of TP508 for treatment of acute traumatic defects and joint surface restoration.
concentrations of Teicoplanin is released evenly and duration of release was satisfactory. In vitro study further demonstrated antibiotic release 28 days after animal implantation. This formulation of Teicoplanin embedded PLGA microspheres appear to be a promising controlled release delivery system for the treatment of bone and joint infections.

Poster No. P171

Optimal Treatment Timing to Rescue Neuronal Cells From Spinal Cord Injury Via Bcl-2 Gene Transfer In Vitro and In Vivo

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Apoptotic cell death occurs following SCI and provides an opportunity for treatment with anti-apoptotic factors (eg. Bcl-2 protein) to potentially improve cell survival. PURPOSE: To investigate the optimum treatment time to deliver the anti-apoptotic protein Bcl-2 via gene transfer with recombinant adeno virus to rescue cells following neural insult. METHODS: Bcl-2 gene transfer was mediated by a recombinant adeno virus using human Bcl-2 oncogene (Adv-Bcl-2). The adenovirus carrying β galactosidase gene (Adv-Bgal) served as a control. The motor neuron cells (NSC-19 cells) were infected with either Adv-Bcl-2 or Adv-Bgal. Seventy-two hours after serum withdrawal, the number of apoptotic cells was counted using Hoechst 33342 staining. For the in vivo animal experiments, a weight drop injury model (10g x 5.0 cm) was used on 81 rats. 1ml virus solution including 2.3 x 108pfu of Adv-Bcl-2 or Adv-Bgal was injected at the epicenter of the injured spinal cord. The degree of cord injury was measured and quantified as the injury index. RESULTS: In vitro: Adv-Bcl-2 infection reduced the apoptotic index (cell death) significantly between 24 hours before (3.5%) and 4 hours after (5.5%) serum withdrawal, the number of apoptotic cells was reduced the number of TUNEL positive apoptotic cells by 28.5% versus 34.2% in controls (P < .01). In vivo: Adv-Bcl-2 injection at 0 H (immediately after injury) significantly decreased the injury index by 24% (P < .01) and significantly reduced the number of TUNEL positive apoptotic cells by 28.5% versus 34.2% in controls (P < .01)

CONCLUSION: Early initiation of Bcl-2 gene transfer produces improved cell and tissue recovery following neural insults by limiting apoptotic cell death.

Poster No. P172

Imaging of Implanted Orthopaedic Hardware Using Tuned-Aperture Computed Tomography® (TACT): A Radiology Evaluation

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INTRODUCTION: TACT three-dimensional images may be a viable alternative when evaluating bony abnormalities around orthopaedic hardware. It utilizes less radiation, costs less than comparable spiral CT imaging, and may provide the clinician with more information that may help evaluate bony architecture.

The FDA has not cleared the drug and/or medical device for the use described in this presentation (i.e. the drug or medical device is being discussed for an “off label” use)
Controlled Delivery of Teicoplanin from an Intraarticular Biodegradable Microparticulate System; In vitro / In vivo Evaluation.

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Key words: Drug Delivery Systems, Teicoplanin

INTRODUCTION
The use of antibiotic impregnated biodegradable carriers has been shown to be a valuable adjunct in the treatment of chronic bone infections. Therefore, the current study was designed to evaluate the controlled antibiotic delivery from a biodegradable microparticulate system to establish levels above the minimum inhibitory concentration for the common causative organisms of bone infections.

METHODS
We used poly lactide-co-glycolide (PLGA 75;25, MW 136000) polymer based microspheres as a biodegradable antibiotic delivery system. Teicoplanin incorporated PLGA microspheres were prepared by emulsion/solvent evaporation process. For characterization of PLGA microspheres and regulation of the release rate, particle size, surface morphology, and drug content was evaluated. (Figure 1)

Agar diffusion method was used for the biological assay of Teicoplanin. Staphylococcus aureus ATCC 25923 strain was inoculated in agar plates. For in vitro release; 30 mg of PLGA microspheres were weighted accurately in separate polypropylene vials for each time point and tubes were placed in a thermostated and bath shaken continuously at 40cpm at 37 C. Samples were taken every 24 hours and after the centrifugation at 10000 rpm, the supernatant was removed and drug content was determined by zone inhibition measurement.

The in vivo study was carried out on three groups of 8-month-old skeletally mature New Zealand white rabbits, weighing around 2.5-3kg (n=18). This delivery system was implanted into the femoral condyle of rabbits through an intraarticular defect of 4mmx10mm. (Figure 2)

The animals were randomized in to three groups. In Group I plain microspheres were implanted into the defect, while in Group II Teicoplanin loaded PLGA microspheres were implanted into the defect, and in Group III Teicoplanin loaded PLGA microspheres were embedded in chitosan gel media. After implantation, regular samples of joint fluid were obtained in 24hr, in days 2, 3, 5 and week thereafter. The antimicrobial activity of this fluid was also measured using an agar gel plate technique as described in vitro analysis.

Fischer’s exact chi-square test was used for statistical analysis.

Further study was designed to evaluate the quantitative analysis of Teicoplanin release. Teicoplanin loaded PLGA microspheres were implanted into the defect to eight rabbit knees. A commercially available immunoanalysis kit (Innofluor® Reagent Set, Opus Diagnostics NJ) and a counter (TDX®, Abbott Laboratories, IL) was used to to determine the in vivo release properties.

RESULTS
Teicoplanin incorporated PLGA microspheres were prepared by 66% yield and average particle size was measured as 29±4.5 μm, while total drug content in microspheres were determined as 1.7%. Differential Scanning Calorimetry (DSC) data showed no interferences formulation between polymer and antibiotic. Scanning Electron Microscopy (SEM) photographs before and after in vitro release studies revealed a uniform distribution and homogeneity of microspheres had a spherical surface. Teicoplanin release from PLGA microspheres continued for 5 weeks. Almost 100% of release was occured on 35th day.
In conclusion, this biodegradable formulation of Teicoplanin embedded PLGA microspheres appear to be a promising controlled release system for Teicoplanin release.

To eliminate the confusing effect of infection, Group II experiment was repeated. In 8 animals 50u.gr Teicoplanin loaded PLGA microspheres were implanted into the defects of rabbit distal femurs. Samples were obtained weekly to allow wound healing and reduce the infection rate. According to the quantity of joint aspirate, agar diffusion method or microdilution technique was used for the biological assay. Results showed an even distribution of microbiologically effective Teicoplanin release in all but one animal (87.5%) during first 4 weeks. In 55.5% of samples at 5th week and, in 42.8% of samples at 6th week we are able to demonstrate MIC levels of Teicoplanin release.

Study for quantitative analysis of Teicoplanin release by immunoanalysis kit did not reveal any information. Counter could not be able to read the released amoutn of Teicoplanin in joint fluid. Commercially available kits arc programmed for serum analysis and we presume that they may not be adequate for intraarticular fluid analysis which has certain different biochemical characteristics.

DISCUSSION AND CONCLUSION

The treatment of musculoskeletal infections with biodegradable materials has the advantages of providing high local levels of antibiotics while maintaining low systemic levels without the need of a second surgery for removal. Various types of carrier materials and antibiotics have been used based on their abilities to achieve controlled bactericidal concentrations. PLGA composite is among the most promising biodegradable biomaterials. It is nontoxic, have FDA approval, elicits minimal inflammatory response, has a controlled resorption rate, and eventually can be resorbed without any accumulation in the vital organs. Teicoplanin is an antibiotic complex recently in clinical use; it is water-soluble, bactericidal, nontoxic to tissue, has a low rate of producing allergen reactions and effective against infections caused by methicillin resistant Staphylococci. In the in vitro part of our study, therapeutic concentrations of Teicoplanin still found during our study. Measurement data suggested that the antibiotic is released evenly and duration of release was satisfactory. In vivo study further demonstrated that the antibiotic release in bone defects maintained constantly 28 days after implantation for all cases and continued to be positive at 42nd day for 42.8% of samples.

In conclusion, this biodegradable formulation of Teicoplanin embedded PLGA microspheres appear to be a promising controlled release delivery system for the treatment of bone and joint infections.

REFERENCES


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