Volume 3

American Academy of Orthopaedic Surgeons®

Proceedings

2002 Annual Meeting

February 13-17

Dallas, Texas

Symposia Handouts

Abstracts of Posters

Abstracts of Papers

Abstracts of Scientific Exhibits

DALLAS TEXAS

Annual Meeting 2002

Poster No. P168

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

James T. Ryaby, PhD, Tempe, AZ (d, e- Orthologic)
Darrell H. Carney, PhD, Galveston, TX
(a- NIH; d,e- Cbrysalis Biotechnology, Inc.)
Rogar A. Crowther, PhD, Galveston, TX
(c- University of Texas Medical Branch; d,
e- Chrysalis Biotechnology)
Jinping Yang, MD, Galveston, TX
Daniel A. Grande, MD, Manhasset, NY

The successful repair of damaged articular canilage remains one of the most difficult challenges in orthopaedic surgen. 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-min diameter, 1.5-2 mm depth) detect was made in the trochlear groove of the femur of young, male New Zealand while 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 ioint surfaces and with poor integration at the junction between repair and native tissue. TP508-treated defects exhibited a predominantlv 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 detects 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.

Poster No. P169

Controlled Delivery of Texcoplanin From an Intra-ArticuIar Biodegradable Microparticulate System: In vitro / In vivo Evaluation

Bulent Atilla, MD, Ankara, TURKEY
(a – ECZACIBASI Scientific Research and Award Fund, Dupost Merck USA)
A. Mazhar Tokgozoglu, MD., Ankara, TURKEY
(a – Eczacıbasi Scientific Research and Award Fund Turkey, Dupost Merck USA)
Sema Calis, PhD, Ankara, TURKEY
(a – Eczacıbasi Scientific Research and Award Fund Turkey, Dupost Merck USA)
Irem Yenice, MS, Ankara, TURKEY
(a – Eczacıbasi Scientific Research and Award Fund Turkey, Dupost Merck USA)
Omur Caglar, MS, Ankara, TURKEY
(a – Eczacıbasi Scientific Research and Award Fund Turkey, Dupost Merck USA)
Meral Ozalp, PhD, Ankara, TURKEY
(a – Eczacıbasi Scientific Research and Award Fund Turkey, Dupost Merck USA)
Hasan Bilgili, PhD, Ankara, TURKEY
A. Mumtaz Alpaslan, MD, Ankara, TURKEY
(a – Eczacıbasi Scientific Research and Award Fund Turkey, Dupost Merck USA)

The use of antibiotic impregnated carriers is valuable in the treatment of hone infections. Current study was designed to evaluate the antibiotic release from a biodegradable microparticulale system. METHODS: Teicoplanin incorporated poly lactide-co-glycolide (PLGA 75;25, M\W 136000) microspheres were prepared by emulsion/solvent evaporation process. Samples were taken every 24 hours and drug content was determined by zone inhibition measurements for Slaphylococcus aureus ATCC 25923 strain in agar gel. The *in riro* study was carried out in to three groups of 8-month-old New Zealand white rabbits (n=18). In Group I plain microspheres were implanted into the femoral condyle intraraticularly. while in Group II Teicoplanin loaded PLGA microspheres, and in Group III Teicoplanin-PLGA microspheres in chitosan gel were implanted. Joint fluid samples were obtained regularly and its antimicrobial activity was measured. RESULTS: *In vitro* Teicoplanin release from PLGA microspheres were continued for 5 weeks. Almost 100% of release was occured on 35th day. *In vivo* analysis revealed concentrations exceeding the MIC for the test organism for two weeks. No statistical significant difference was detected between Groups II and III. *(p>* 0.05). Forty percent of samples demonstrated antimicrobial activity in at the fourth week. DISCUSSION AND CONCLUSION: PLGA composite is among tlie most promising biodegradable materials for the treatmant of muskuloskeletal infections. In the *in vitro* part of our study, therapeutic

concentrations of Teicoplanin is released evenly and duration of release was satisfactory. *In tiro* study further demonstrated antibiotic release 28 days after animal implantation. This formulation of Teicoplanin embedded PLGA microspheres appear 10 be a promising controlled release delivery system for the treatment of bone and joint infections.

Poster No. P170

Tissue Engineered Human Articular Cartilage Produced From Knee Biopsy Specimen-Alginate-Recovered-Chondrocyte Method and Osteogenic Protein-1

Koicbi Masuda, MD, Chicago, IL.
Tcitsnya Miyazaki, MS, Chicago, IL.
Brian J. Cole, MD, Chicago, IL.
Howard S. An, MD, Chicago, IL.
(a - Stryker Biotech. Inc.; e - Articular Engineering LLC)
Michael]. Hejva, MD, Chicago, IL.
Eugene Thonar, PhD, Chicago, IL.

We have developed a novel, scaffold free culture method (alginate-recovered-chondrocyte method, ARC, method) for the production of cartilaginous tissue *in vitro* that does not require exogenous matrices. The purpose of this study was to test whether this type of tissue engineered cartilage could be produced from a limited amount of human cartilage for aulograft application. METHODS: Human cartilage (less than 113 mg in each case) was obtained from the intercondylar notch of the knee joint of patients (6 cases, age 18-58) undergoing notchplasty for anterior cruciate reconstruction. The cartilage was digested and the released cells expanded in monolayer culture (two or three passages) in DMEM/F12 + 20% FBS and 200 ng Osteogenic protein-1 per ml. Cells were cultured in alginate gel for 14 to 36 davs and he cells and their associated matrix were recovered, and seeded onto a tissue culture insert in the same media. At the end of the culture period, the weights of the tissues were measured. The tissues were examined histologicaly and biochemically. RESULT: The cells in the monolayer proliferated over 30 times during the 3 passages. Using (lie ARC. method to engineer cartilage after the cell expansion, easy to handle cartilage (23 mm diameter and 0.8 mm thickness) was routinely obtained within 8 weeks (i.e. 4 weeks in alginate and 4 weeks on tissue insert). Biochemical analyses of the tissue showed a stable chondrocytic phenotype. DISCUSSION AND CONCLUSIONS: The ARC method will enable us to engineer the transplantable cartilaginous tissue after cell expansion and be useful for autologous cartilage repair.

Poster No. P171

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

Yasutsugu Yukawa, MD, St. Louis. MO
Juren Lou, MD, St. Louis, MO
Naoshi Fukui, MD, St. Louis, MO
Lawrence G. Lenke, MD, St. Louis, MO

Apoptotic cell death occurs following SC1 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 adenovirus to rescue cells following neural insult. METHODS: Bcl-2 gene transfer was mediated by a recombinant adenovirus using human Bcl-2 oncogene (Adv-Bcl-2). The adenovirus carrying ß 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 afler 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 (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 apopiolic cells by 28.5% versus 34.2% in controls (P < .01) CONCLUS1ON: 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 Computec Tomography® (TACT): A Radiology Evaluation

David C. Pollock, MD, Winston-Salem, NC
Bryon A. Dickerson, MD, Winston-Salem, NC
Felix S. Chew, MD, Winston-Salem, NC
Richard L. Weber, Winston-Salem, NC
(a- instrumentarium Imaging.)

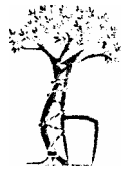
INTRODUCTION: TACT three-dimensional images may be a viable alternative when evaluating bony abnormalities around orthopaedic hardware. It utilizes less radiation, costs less than comparable spiral CT imaging, and may provide the clinician wihe more information that may help evaluate bony architectu

Controlled Delivery of Teicoplanin from an Intraarticular Biodegradable Microparticulate System; In vitro / In vivo Evaluation.

Bulent Atilla, MD [(1)], A. Mazhar Tokgozoglu, MD [(1)], Sema Calis, PhD [(2)], Irem Yenice, MS [(2)], Omur Caglar, MD [(1)] Meral Ozalp, PhD [(3)], H Bilgili, PhD [(4)] , A. Mumtaz AIpaslan, MD [(1)].

[(1)] Hacettepe University Faculty of Medicine Department of Orthopaedics and Traumatology, 06100 Ankara TURKEY,
[(2)] Hacettepe University Faculty of Pharmacy Department of Pharmaceutical Technology, 06100 Ankara TURKEY,
[(3)] Hacettepe University Faculty of Pharmacy Department of Pharmaceutical Microbiology, 06100 Ankara TURKEY,
[(4)] Ankara University Veterinary Faculty, Ankara TURKEY

Hacettepe University
Ankara, 1967



Dept. of Orthopaedics
and Traumatology

Key words: Dru? 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 microparticulat 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 systems Teicoplanin incorporated PLGA microspheres were prepared by emulsion/solvent evaporation process. For characterization of PLG 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

Figure 1. Scanning electronmicroscopy photographs of Teicoplanin loaded microspheres before (a), and after (b) antibiotic release.

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 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 wei implanted into the defect to eight rabbit knees. A commercially available immunoanalysis kit (Innofluor® Reagent Set, Opus Diagnostic 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 im, while tot drug content in microspheres were determined as 1,7%. Differencial Scanning Calormetry (DSC) data showed no interferences formulation between polymer and antibiotic. Scanning Electron Microscopy (SEM) photographs before and after in vitro release studies revealed that microspheres were homogenous and had a spherical surface. Teicoplanin release from PLGA microspheres were continued for 5 weeks. Almost 100% of release was occured on 35th day.

Figure 2. Lateral femoral condyle of rabbits was exposed and a standart 4x10 mm transverse hole was drilled (a), Teicoplanin loaded beads were implanted in this defect (b), and layers were closed (c).

In vivo analysis revealed that the drug concentration exceeded the MIC for the test organism for two weeks without inducing serum toxic levels. There was no statistical significant difference detected between Groups II and III. (p> 0.05). **(Figure 3)** Samples from the Group I presented no antimicrobial activity. At the end of second week, 86% of animals in Group II and III were complicated with wound infection and 92% of them were healed without any treatment at the fourth week follow-up. Further testing with agar gel plate demonstrated antimicrobial activity in 40% of samples at the fourth week after the infection has subsided.

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 microbioiogically 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 samplesat 6th weekwe are able to demonstrate MIC levels of Teicoplanin release.

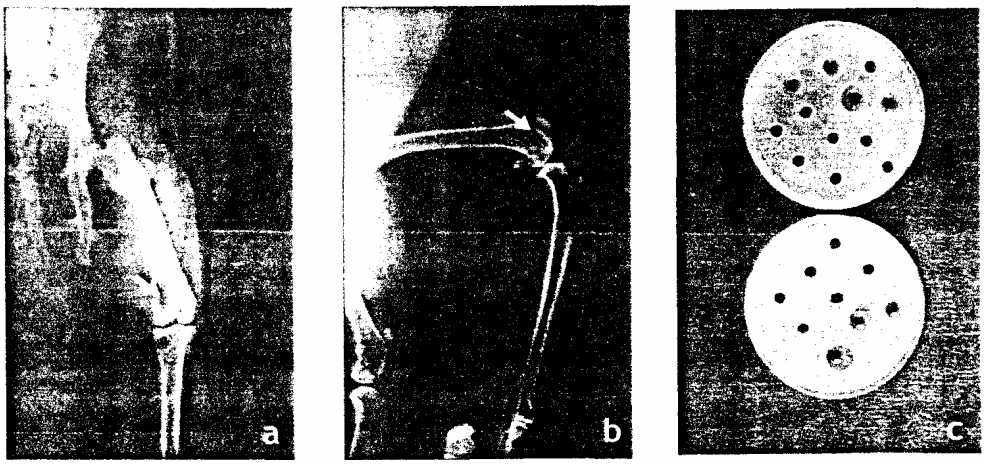


Figure 3. AP and LAT plain radiograms of rabbit distal femurs 10 days after implantation (a and b), and clearance zones around joint aspiration fluid samples in Agar, for microbiological assessment of Teicoplanin release at the end of the 2nd week.

Study for quantitive analysis of Teicoplanin release by immunoanalysis kit did not reveal any information. Counter could not be able to read the released amonut 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 muskuloskeletal 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 with no 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 35 days. 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 PLGAmicrospheres appear to be a promising controlled release delivery system for the treatment of bone and joint infections.

REFERENCES

1. Schmidt C, Wenz R, Nies B, Mool F. Antibiotic in vivo/vitro release, h,stocompatabi'ityand btodegradation of gentamycin implants based on lactid acid polymers and copolymers. J Control Rel;37;E3-94 1995
2. Atkins TW, Peacock SJ, Yates DJ. Incorporation and release of Vancomycin from poly (D, L-lactide-co-glycolide) Microspheres. J Microencap, 15,31-44; 1988
3. Athanasiou,KA,Agrawal M, Ba-berA,BurkhartSS. Orthopaedic applications for PLA-PGA biodegradable polymers. J Artroscopic Re! Surg. 14-.726-37; 1988
4. Blaiser RD, Bucholz R, Cole W, Johnson LL, Makela E. Bioresorbable implants; applications in orthopaedic surgery *Instructional Course Lectures* (Ed. D S Springfeld);531 -46; 1997
5. Garvin KL, Miyano JA, Robinson D, Giger D, Novak J, Radio S. Polylactide/polyglycolide antibiatgc implants in thetreatmnet of osteomyelitis. J Bone Joint Surg. 76(Am);1500-6; 1994
6. Aimin,C,Chun!in,H., Juliang,B,Tinyin.2,2hichao, D. Antibiotic loaded chitosan bar; an in vivo study of a possible treatment for osteomyelitis. Clin.Orthop.ReEat. Res. ,366 ,239-247,1999
7. Kas H.S Chitosan; properties ; preparations and application to microparticulate systems, J- Microenc. 14,689-711, 1997
8. DacQuetV.VarletA.TandoganRN.TahonM.FournierL, Jehl F, Monteil H., BascoulereeG. Antibiotic impregnated plaster of pans beads: trials with teicoplanin. Clinical Orthop. Ret. Res. ,240-249,1992
9. Tuncay M., Call} S, Kas H.S, Peksoy I, HincalAA. Dicloferac sodium incorparaled PLGA(50,50) microspheres : formulation considerations oral fn vitro / in vivoevalution. Int.J. Pharrn., 195,179-188, 2000
10. Wei G. Kotoura Y. ,0ka M., YamamuraT, Wada R., Hyon S. Ikada V. Abioabsorbable delivery system for antibiotic treatment for osteomyelitis.J Bone Joint Surg., 73-B,246-252,1991
11. Shinto Y,UchidaA.,KorkusuzF,ArakiN,OnoK. Calcium hydroxyapatite ceramic used as a delivery system for antfciotics.J. Bone Joint Surg. (Br), 74-B,600-604,1992
12. Brogden R.N, Peters DH. Teicopianin; a reappraisal of its antimicrobial activity, pharmocokinetics propertiesano therapeutic potential. Drugs,40 (3), 449-486,1994.

Acknowledgement

This study was supported by Eczacibasi Scientific Research and Award Fund (Turkey). Authors wish to acknowledge Dupont Merck (USA) fo the generous supply of PLGA.

Address for Correspondence:
Bulent Atilla, MD.
Hacettepe University, Faculty of Medicine, Department of Orthopaedics and Traumatology
06100 Samanpazan, Ankara, Turkey
e-mail: batilla@hacettepe.edu.tr Fax: (90) 312 310 01 61