



2001/2

# AOA RESEARCH FOUNDATION LTD APPLICATION FOR RESEARCH GRANT

Applicants should read the Instructions to Applicants before completing this form

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**Project Title:**

Effect of Bisphosphonates on a Spinal Fusion Model in NZW Rabbits

**Details of Chief Investigator:**

Title, Initials and Surname: Dr David G Little  
Appointment: Head  
Department: Orthopaedic Research Unit  
Children's Hospital at Westmead  
Locked Bag 4001  
WESTMEAD NSW 2145

Academic Qualifications (Indicate conferring institutions and dates):

MBBS	University of Sydney	1986
FRACS(Orth)	RACS	1995
FA(Orth)A	AOA	1998

Hours/week devoted to this project: 4  
Hours/Week devoted to other projects: 12

**Details of Associate Investigators:**

(1) Title, Initials and Surname: Dr Andrew Cree  
Appointment: Visiting Medical Officer  
Department: Children's Hospital at Westmead

Academic Qualifications (Indicate conferring institutions and dates):

MBBS	University of Sydney	1990
FRACS(Orth)	RACS	1998

Hours/week devoted to this project: 4  
Hours/Week devoted to other projects: 2

(2) Title, Initials and Surname: Dr Elisabeth Goergens  
Appointment: Orthopaedic Fellow  
Department: Orthopaedic Research Unit  
Children's Hospital at Westmead (as of January 2002)

Academic Qualifications (Indicate conferring institutions and dates):

MD	University of Heidelberg	1995
Facharztin für Orthopädie	Arztekammer Hamburg	1998

Hours/week devoted to this project: 20  
Hours/Week devoted to other projects: 10

**Institution at which research will be conducted:**

Children's Hospital at Westmead  
University of Sydney

**Institution which will administer the grant:**

Children's Hospital at Westmead

**EXPERIMENTS ON HUMAN SUBJECTS OR ANIMALS**

(Delete inappropriate option)

Does the project involve experiments on human subjects? NO

If Yes, has the Ethics Committee of the Institution concerned approved the project and certified that it conforms to the general principles set out in the NH&MRC Statement on Human Experimentation?

Does the project involve experiments on animal subjects? YES

If Yes, has the Ethics Committee of the Institution concerned approved the project and certified that it conforms to the general principles set out in the NH&MRC Statement on Animal Experimentation?

NOT YET

Signature of Chief Investigator:

*D Little*

Date: 16/5/01

**CERTIFICATION OF HEAD OF DEPARTMENT**

I certify that this project is appropriate to the general facilities in my Department and that I am prepared to have the project carried out in my Department.

Title and Name: *DR DAVID LITTLE*

Department: *Orthopaedic Research Unit*

Signature: *D. Little*

Date: 16/5/01

**CERTIFICATION OF HEAD OF INSTITUTION OR NOMINEE**

I certify that this request satisfies all the requirements of the institution and that the classifications quoted for personnel are in accordance with practice at this institution.

Title and Name: *Prof. R. K. OATES*

Appointment: *CEO*

Signature: *[Signature]*

Date: 17.5.01

**BUDGET**

Detailed Budget Items	Priority	Amount Requested (\$)	Office Use Only
30 SPF NZW rabbits	High	3,000	
QCT Scans	High	2,000	→ salary.
Scientific Officer, \$25.00 / hour, 80 hours	High	300	
Radiographs 30 @ \$10.00	High	400	
Sterile Drapes, Blades, running costs	High	1,200	
Zoledronic Acid	High		
<b>TOTAL</b>		<b>6,900</b>	<i>4,900 disposable</i>

Note: No funds sought for fellow's salary. Osteoset or other calcium sulphate bone graft substitute will be supplied by company.

**OTHER RESEARCH SUPPORT**

Currently Held Funding:			
Project Title	Source	Final Year	Funding (\$)
Pharmacological and Mechanical Manipulation of a Distraction Osteogenesis Model in Skeletally Immature Rabbits	Children's Hospital Fund	2001	\$145,000.00 over three years
Requested Funding for Coming Year			
Project Title	Source	Final Year	Funding (\$)
Can bisphosphonates prevent collapse of the femoral head in osteonecrosis?	Financial Markets Foundation for Children	2002	\$41,053.84

## **AIMS OF THE PROJECT:**

List the specific aims and potential significance of the project. If hypotheses are to be tested, they should be clearly stated.

This study will analyse the effect of bisphosphonates in potentiating the amount of new bone formation, its mineralisation and mechanical properties in a NZW rabbit spinal fusion model. The graft substitute, Osteoset, will be used and the effect of augmenting this with bisphosphonate administration analysed. Three groups of animals tested will consist of:

Vehicle (VEH):	Osteoset putty alone
Systemic (SYS):	Osteoset putty and two doses of intravenous bisphosphonate
Local (LOC):	Osteoset putty admixed with bisphosphonate and applied locally at surgery

This experiment will specifically test the following hypotheses:

1. Two dose bisphosphonate administration enhances spinal fusion healing when utilising a standardised amount of putty
2. Locally administered bisphosphonate administration enhances spinal fusion healing when utilising a standardised amount of putty

## **ETHICAL IMPLICATIONS OF THE PROJECT: EXPERIMENTS ON HUMANS AND ANIMALS**

The Chief Investigator has performed numerous experiments on the effects of bisphosphonates in distraction osteogenesis in a similar NZW rabbits model. The intervention involves a single level bilateral L5-L6 fusion. As this model involves a simple approach to the spine, the animal is in little pain and moves about the cage freely after 24 hours. Euthanasia is performed humanely with an intravenous barbiturate.

The study will be subject to approval by the Westmead Animal Ethics Committee in line with NHMRC guidelines.

## BACKGROUND, RESEARCH PLAN, JUSTIFICATION OF BUDGET AND RELEVANT PUBLICATIONS

Only published papers and papers accepted for publication in refereed journals are to be listed. Abstracts should not be included. Ensure that sufficient detail is provided in the Research Plan for assessors to understand the proposal. Up to 5 additional A4 pages may be attached for this section.

Under "**Background**", the problem which is to be addressed should be defined, in terms of work done by others or by yourself. References to appropriate published papers should be included.

In "**Research Plan**" the methods to be used should be described, and the general plan of the experiments or observations, in such a way as to demonstrate how the hypothesis advanced under "Aims" will be tested.

The "**Budget**" should be justified by briefly describing why the support requested is essential to the project.

Under "**Relevant Publications**" should be listed those publications of the chief investigators which bear directly on this project. These should not normally exceed 10 in number

### Spinal Fusion

Spinal fusion is a very common orthopaedic procedure performed to correct or prevent deformity and to relieve pain and disability in children. Adult patients undergo spinal fusions for similar reasons.

Pseudarthrosis or non-union is a common complication of this procedure. Internal fixation has improved fusion rates, but failures still occur, prompting a large body of investigative work into improving fusion rates. Current reported fusion rates are around 75% to 90%, ie up to one quarter of operations fail to produce spinal fusion<sup>1,2</sup>. This is even more of a concern in some paediatric conditions such as neurofibromatosis and spondyloepiphyseal dysplasia, where spinal deformity occurs commonly and attempts at fusion are frequently unsuccessful.

Autogenous bone grafting remains the gold standard, and is often, but not always successful. Morbidity related to the donor site at the iliac crest has been estimated as 30%, and is quite painful in most cases. Autograft, usually taken from the ilium, is often in short supply, especially in children. Thus, there is much research activity into developing bone graft substitutes or extenders, in order to reduce this morbidity.

Graft substitutes are meant to be both osteoinductive and osteoconductive, while graft extenders usually provide an osteoconductive scaffold but require autograft to provide the osteoinductive component.

The simplest graft extenders are calcium crystal complexes and include coralline hydroxyapatite (Pro-Osteon®), calcium sulphate (Osteoset®) and Tricalcium Phosphate cement (Norian®). The newer approaches involve the inclusion of various bone promoting growth factors in a delivery medium. Demineralised bone matrix in various forms, gel, putty or sheet (Grafton®, DynaGraft®, Osteofil®, AlloGro® etc) are available. The demineralised bone still contains some of the mediators implicated in bone healing. There remains a theoretical concern about disease transmission or allergic problems with such a material. Demineralised bone matrix is a graft extender, allowing small amounts of graft to go further, but has not increased fusion rates in most studies<sup>3</sup>. One recent study suggested the putty form of Grafton® leads to spinal fusion alone in experimental animals<sup>4</sup>. Transmission of infectious diseases by the use of allografts is possible, if protocols designed to prevent this break down.

Refined human products, such as Bone Morphogenetic Protein 7 (OP1), are available but very expensive. Other products using TGF- $\beta$  are in development. Research is also underway into the use of gene therapy, exploiting an adenoviral vector to stimulate mesenchymal stem cells to produce Bone Morphogenetic Protein 2 (BMP 2)<sup>5</sup>. Such techniques involve harvesting of bone marrow cells and their growth in tissue culture. This is time consuming, labour intensive, expensive and probably of little relevance in clinical practice. Our preliminary results on the up-regulation of growth factors in a bisphosphonate treated distraction osteogenesis model indicate that some of these growth factors may be elevated *in vivo* by bisphosphonates. More simple bone graft substitutes, such as calcium sulphate, should prove cheaper. However the more simple the substitute the less likely it is to be osteoinductive.

Although it increases fusion rates, internal fixation can also reduce the amount of fusion mass bone that forms as movement is reduced. Movement is a powerful stimulator of bone production. We have shown in our distraction osteogenesis studies (below) that we can increase the amount of new bone formation with bisphosphonates even in the presence of rigid fixation.

A reliable NZW rabbit model of spinal fusion shows 66-73% success rate in obtaining fusion by manual testing when autogenous bone is used<sup>3</sup>. The rate of fusion may be lower when analysed with CT scan (as we intend to do). We aim to use this model to assess if bisphosphonates can add to the osteogenic potential of a standard amount of non-organic bone graft substitute - calcium sulphate (Osteoset).

We plan to investigate the use of locally available autogenous bone, which in clinical practice could be harvested at the site of operation, admixed with calcium sulphate bone graft substitute. By admixing the graft with absorbable calcium sulphate putty and augmenting the osteogenic potential with a bisphosphonate, we could considerably improve the current clinical situation, with much less cost than using gene products such as OP1, TGF- $\beta$  or implanting demineralised bone matrix.

## Bisphosphonates and Bone Healing

Our group has performed several successful experiments utilising bisphosphonates to improve new bone formation and reduce stress-shielding osteoporosis in a distraction osteogenesis model in the NZW rabbit. This work is about to move into the clinical trial phase. This experiment aims to lay further groundwork in the area of bone defect healing and spinal fusion. Bisphosphonates have not been previously utilised in this context, and we hypothesise that bisphosphonates could improve the success rates in these circumstances.

Briefly, in trials of pamidronate in an immature (8-week-old) rabbit model of distraction osteogenesis, a single dose of 3mg/kg at the time of surgery increased area BMD by an average of 39-43% ( $p < 0.05$ ) in the regenerate and adjacent bone over controls<sup>6</sup>. Preliminary histological observations in 10 rabbits indicated increased osteoblast and decreased osteoclast presence in both regenerate and adjacent bone in the pamidronate-treated group. A single 1mg/kg dose showed a 32% ( $p < 0.05$ ) increase in peak load tested by four point bending.

We have recently performed trials with zoledronate in distraction osteogenesis that are even more promising<sup>7</sup>. The cross sectional area of new bone formed in the regenerate was increased by 49% in the zoledronate group versus controls and by 59% in the re-dosed zoledronate group (Fig. 1). The bone mineral content (BMC) of the regenerate was increased by 93% in the zoledronate group versus controls and by 111% in the re-dosed zoledronate group. Bone mineral accrual was 2.5 times faster in the treated groups in the first two weeks after distraction.

Localised disuse osteoporosis in the bone adjacent to the distraction site was abolished; in fact there was a significant increase in bone formation at these usually vulnerable sites. Mechanical testing of the tibia in four-point bending showed increases in peak load of 29% for the single dose group and 89% for the re-dosed group (Fig 2). This information clearly demonstrates a beneficial effect of zoledronate on bone healing in distraction osteogenesis. We are hopeful we can achieve similar results in this experiment, opening up a whole new field in filling bone defects and obtaining spinal fusions.

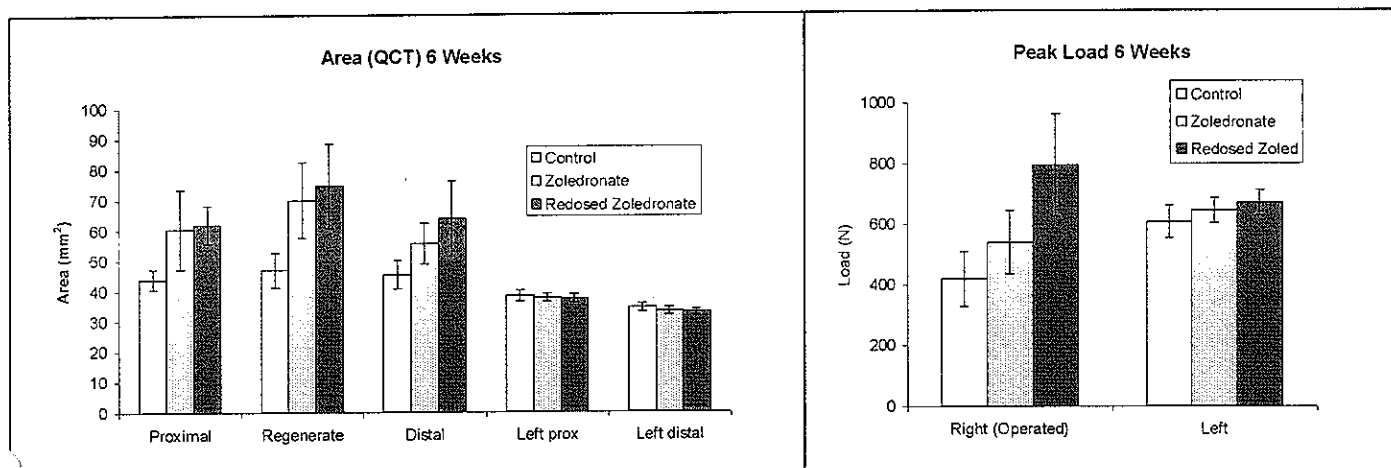


Fig 1

Fig 2

## Choice of bisphosphonate

Zoledronate (Novartis), a third generation bisphosphonate, has a side chain with an imidazole ring. A more potent inhibitor of osteoclasts than earlier bisphosphonates, zoledronate has little negative effect on bone mineralisation *in vitro* and the largest therapeutic ratio between the desired inhibition of calcium resorption and the unwanted inhibition of mineralisation *in vitro* of all current bisphosphonates<sup>8</sup>. Zoledronate also has a lower risk of nephrotoxicity than other bisphosphonates, and our rabbit nephrocalcinosis data shows zoledronate to be safer<sup>9,10</sup>. Pamidronate is given as a two-hour infusion; zoledronate can be administered in five to twenty minutes. Other new potent bisphosphonates similar to zoledronate are now also available, namely risedronate and ibandronate. Risedronate has been shown to potentially improve outcomes in osteoporosis, with reduced fracture rates. It is a very similar formulation to zoledronate, but can be given orally. We plan to use either zoledronic acid (zoledronate) or risedronate, depending on availability. They are very similar compounds owned by different companies.

## Research Plan

### Animal Model

We will use an 8-week-old NZW rabbit model (Boden). This involves a single level bilateral L5-L6 fusion. Boden and colleagues have previously assessed that non-organic bone graft extenders are insufficient to provide spinal fusion alone, and advocate more expensive bioactive substitutes, such as BMP's and even more novel gene products<sup>11</sup>. We therefore expect that many of the vehicle osteoset group will not fuse. Fusion of the bisphosphonate treated animals would constitute an advance.

As this model involves a simple approach to the spine, the animal is in little pain and moves about the cage freely after 24 hours. State of maturity will be confirmed by measurement of testicular weight at culling.

## **Animal Care**

Rabbits will be housed in the Westmead Animal Care Facility in a battery containing 3 double cages with indirect wood shaving bedding. Diet will be rabbit pellet and water ad libitum. Animals are housed one per cage.

## **Premedication / Sedation**

IM Ketamine 15 mg/kg, Xylazine 4mg/kg given in combination 10 minutes before surgery.

## **Anaesthesia**

Inhaled Halothane 2% and Oxygen 1L/min.

## **Infusion**

Zoledronate (or Risedronate) 0.1 mg/kg diluted to a concentration of 1mg/100ml will be given over fifteen minutes at the time of surgery via an ear vein. Saline only is given to the vehicle control group. These doses are repeated in both groups at two weeks.

## **Surgery**

Surgery will take place in an animal theatre. The operative field will be prepared by shaving with clippers, disinfected with povidone iodine 4% w/v in 70% alcohol. The animal will lay prone, allowing good access to the lumbar spine.

A midline approach to the spine will be made at the L5-L6 level. The bed of the L5-L6 posterolateral gutter will be prepared with a burr to expose underlying cancellous bone

0.5 ml of Osteoset calcium sulphate putty will be placed in each posterolateral gutter of the spine. The Osteoset will be rolled out to a thickness of 2.5 mm and a width of 10 mm and length of 20 mm.

The wound will be irrigated with N/Saline and then with a solution containing 600mg of Benzyl-penicillin to minimise the risk of infection. Local anaesthetic (bupivacaine) will be infiltrated into the wound. The wound will then be closed with a dissolvable suture.

## **Recovery**

The rabbits will receive Buprenorphine 0.05 mg/kg at the end of surgery and every 12 hours post-operatively as required.

## **Euthanasia**

The animals will be culled with IV Lethobarb 150 mg/kg at the end of the experiment.

## **Radiographic Analysis**

The spine will be harvested through the sacro-iliac joints and from L2 to S3. This segment will also be radiographed as above.

## **pQCT Scanning**

The disarticulated spinal segments are stripped of muscle and analysed using a Stratec XCT-960A pQCT scanner and analysis software (Stratec Medizintechnik GmbH, Pforzheim, Germany). The spinal segment will be analysed axially at the L5 and L6 level to assess the cross sectional area of the bone mass in the posterolateral gutter. A sagittal longitudinal scan will also be performed to confirm union between the transverse processes. If the gap between the transverse processes is bridged, it will be classified as fused.

## **Mechanical Testing**

All specimens will be frozen in saline-soaked gauze at -70C. The spinal complexes will be placed in resin such that only the fused segment is free of the resin. The construct will be tested in torsion to failure at a constant rate of 5 degrees per minute.

Data for peak torque, stress and strain will be calculated and compared for both the bisphosphonate and vehicle groups.

## **STATISTICAL METHODS AND POWER**

### **Power**

The experimental groups compared in this study will be the spines of rabbits treated with vehicle only (VEH10 rabbits, 20 fusions) and those treated with vehicle and systemic bisphosphonates (SYS 10 rabbits, 20 fusions) and those treated with local administration of bisphosphonates (LOC 10 rabbits, 20 fusions).

The number fused sides in each group will be assessed for differences using Fisher's exact test. Boden et al noted no fusion with hydroxyapatite calcium substitute. Assuming by chance 4 of 20 segments fuse in the VEH group, an increase in the fusion rate to 12 out of 20 in either bisphosphonate group either SYS or LOC, would be statistically significant.

Data for fusion mass cross-sectional area and mechanical testing outcomes will also be collated, previous data is lacking to make an accurate power analysis.

### **Randomisation**

Two runs of 15 animals will be performed. Rabbits will be randomised such that 5 animals in each of the VEH, SYS and LOC groups are included in each run, to allow for variations in size and health of different litters.

### **Data Analysis**

The spinal CT data will be analysed for cross-sectional area of new bone formation in the posterolateral gutter at the L5-L6 disc level. These data will be compared by student t test if normally distributed, by rank test if not normal. BMD and BMC of the

fusion mass will also be recorded. The sagittal scans will be assessed as united or not united by the presence or absence of bridging across the transverse processes

The peak torque values will be compared in the same fashion as and cross-sectional area.

#### **Personnel**

This study will be carried out by the Orthopaedic Research Unit at The Children's Hospital at Westmead, headed by Dr David Little. Dr Andrew Cree, Spinal Surgeon, will be a co-chief investigator. Orthopaedic research fellows rotate six monthly through the Unit. These fellows are funded by charitable donation. The fellows will carry out the surgical procedures under the supervision of Dr Cree and care for the animals with the aid of animal house staff, and assist in the mechanical testing at the University of Sydney as part of their research training. Dr Little supervises the fellows. Mechanical Testing is co-supervised by Dr Lynne Bilston of the Orthopaedic Research Unit and Department of Mechanical Engineering, University of Sydney.

#### **Timetable**

15 rabbits can be housed in each 8-week cycle (2 weeks acclimatisation, 6 weeks experimental). Two cycles are planned the six-month period from January to July 2002. The operative part of the project will be completed by July 2002. DXA, pQCT and mechanical testing occur soon after culling and the analysis of these only takes one to two months.

#### **Outcomes and Significance**

Healing of spinal fusions is a common problem facing orthopaedic surgeons. Many investigations using many biologically active substances have been performed. Our work on bisphosphonates leads us to believe that the bone forming potential of small amounts of autogenous graft and graft substitutes can be improved by bisphosphonate action. Improving healing and fusion rates is desirable, and achievable in the short term.

A positive outcome in this controlled animal experiment would lay the foundations for carefully constructed clinical trials of bisphosphonates in such situations. Local administration of bisphosphonates admixed with the putty and bone graft mixture could lead to a simple, relatively cheap and safe way of augmenting spinal fusions in clinical practice.

#### **Justification of Budget**

The NZW rabbits requested, x-rays, CT scans and \$400 running costs are all intrinsic to the project. The Orthopaedic Research Unit will fund the salaries of Orthopaedic personnel involved in the study, and the Hospital will absorb the costs of housing the animals.

We will have the calcium sulphate donated by a company or use bulk chemical stock, which is not costly.

## Relevant Publications

- 1 Snider RK, Krumwiede NK, Snider LJ, Jurist JM, Lew RA, Katz JN. Factors affecting lumbar spinal fusion. *J Spinal Disord.* 1999;12:107-14.
- 2 Jenkins LT, Jones AL, Harms JJ. Prognostic factors in lumbar spinal fusion. *Contemp Orthop.* 1994 Sep;29(3):173-80.
- 3 Morone MA, Boden SD. Experimental posterolateral lumbar spinal fusion with a demineralized bone matrix gel. *Spine* 1998 15:23:159-67
- 4 Martin GJ, Boden SD, Titus L, Scarborough NL. New formulations of demineralized bone matrix as a more effective graft alternative in experimental posterolateral lumbar spine arthrodesis. *Spine* 1999;24:637-45
- 5 Riew KD, Wright NM, Cheng S, Avioli LV, Lou J. Induction of bone formation using a recombinant adenoviral vector carrying the human BMP-2 gene in a rabbit spinal fusion model. *Calcif Tissue Int.* 1998;63:357-60.
- 6 Little DG, Cornell MS, Cowell CT, Briody J, Arbuckle S, Cooke-Yarborough C. Intravenous pamidronate reduces osteoporosis and improves regenerate formation during distraction osteogenesis in immature rabbits. In Press 2001 *J Bone Joint Surg (Br)*.
- 7 Little DG, Smith NC, Williams P, Briody J, Cowell CT. Effect of Zoledronate in distraction osteogenesis in rabbits. Presented at the Japanese Orthopaedic Research Society, Kyoto, September 2000.
- 8 Green JR, Muller K, Jaeggi KA. Preclinical pharmacology of CGP 42446, a new, potent heterocyclic bisphosphonate compound. *J Bone Miner Res* 1994; 9:745-751.
- 9 Green JR, Seltenmeyer Y, Jaeggi KA, Widler L. Renal tolerability profile of novel, potent bisphosphonates in two short-term rat models. *Pharmacol Toxicol* 1997; 80:225-30
- 10 Williams P, Smith N, Cooke-Yarborough C, Little DG. Reduced nephrocalcinosis with zoledronate in a distraction osteogenesis model in rabbits. Unpublished data.
- 11 Boden SD. Biology of lumbar spine fusion and use of bone graft substitutes: present, future, and next generation. *Tissue Eng.* 2000;6:383-99.