The role of the osteoclast during endochondral ossification in a rat fracture model

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Certificate of authorship

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree as fully acknowledged within the text.

I also certify that the thesis has been written by me, any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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Acknowledgements and Dedication

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Abbreviations

ALN	Alendronate
αvβ3	Alpha V beta 3
ANOVA	Analysis of Variance
ATP	Adenosine triphosphate
bFGF	Basic fibroblastic growth factor
BMC	Bone mineral content
BMD	Bone mineral density
BMU	Bone remodelling unit
BP	Bisphosphonate
BPs	Bisphosphonates
BV/TV	Bone volume/ total volume (%)
ClC-7	Chlorine channel - 7
Col X	Collagen type X
CSF-1	Colony stimulating factor-1
CMC	Carboxymethyl cellulose
CTX	C-terminal telopeptide cross-links
DEPC	Diethylpolycarbonate
DEXA	Dual energy X-ray Absorbtiometry
DMSO	Dimethyl sulfoxide
ECM	Extracellular matrix
EDTA	Ethlyenediaminetetra Acetic Acid (disodium salt)
ELISA	Enzyme-linked immunosorbent assay
FBS	Foetal Bovine Serum
FPPS	Farensyl diphosphate synthase

GTP	Guanosine triphosphate
HD	High dose
HS	Heperan sulfate
HSPG	Heperan sulfate proteoglycans
ia/ia	Incisor absent rat
ICTP	Cross-linked carboxyterminal telopeptide of type I collagen
Ihh	Indian hedgehog
IV	Intravenous
KO	Knockout
LD	Low dose
M-CSF	Macrophage colony stimulating factor
µCT	Micro computerised tomography.
Mins	Minutes
MMA	Methylmethacrylate
MMP	Matrix metalloproteinase
MMP-9	Matrix metalloproteinase 9
MMP-13	Matrix metalloproteinase 13
mRNA	Messenger ribonucleic acid
N-BP	Nitrogen containing Bisphosphonate
NLM	Normal littermate
Oc	Osteocalcin
oc/oc	osteosclerotic mouse
OI	Osteogenesis Imperfecta
op/op	osteopetrotic mouse
OPG	Osteoprotegerin

OPN	Osteopontin
OVX	Ovariectomised
PBS	Phosphate buffered saline
P-C-P	Phosphorous-carbon-phosphorous
PCNA	Proliferating cell nuclear antigen
PECAM	Proliferating endothelial cell adhesion marker
PFA	Paraformaldehyde
PLGA	Poly L-lactide-co-glycolide
Ptc	Patched 1
PTH	Parathyroid hormone
PTHrP	Parathyroid hormone related protein
QCT	Quantitative computerised tomography
RANK	Receptor-activator of nuclear factor kappa beta
RANKL	Receptor-activator of nuclear factor kappa beta ligand
RNA	Ribonucleic acid
ROI	Region of Interest
RT	Room temperature
SC	Subcutaneous
SD	Standard deviation
SE	Standard error
Tb.N	Trabecular number
Tb.th	Trabecular thickness
TGFβ	Transforming growth factor beta
tl/tl	Toothless rat
TRAP	Tartrate resistant acid phosphatase

VEGF	Vascular endothelial growth factor
wt/het	wild type / heterozygous
ZA	Zoledronic Acid

Abstract

Bisphosphonates (BPs) are the most common treatment for osteoporosis, due to their powerful ability to inhibit osteoclastic bone resorption. They are also being investigated to augment callus production during fracture healing, however, concerns exist as to the effects of BPs during both initial fracture union and hard callus remodelling. Endochondral ossification during fracture repair is a critical process leading to initial union, and is assumed to be dependent on osteoclast function. Hard callus remodelling, known to be dependent on osteoclast function, is important to the completion of bone repair.

The role of osteoclasts during initial endochondral fracture union was investigated using the BP zoledronic acid (ZA) and in a genetic model of osteoclast inactivity, the incisor absent (*ia/ia)* rat. In addition, the effect of differing ZA treatment regimes on hard callus remodelling was investigated using both Bolus and Weekly ZA dosing. A Bolus of 0.1mg/kg ZA or 5 Weekly doses of 0.02mg/kg ZA or Saline were administered commencing 1 week post surgery in a rat femoral fracture model. Femoral fractures were also produced in *ia/ia* rats. Examinations were performed up to initial union and throughout callus remodelling.

ZA treatment did not alter the rate of endochondral fracture union. All fractures united by 6 weeks, with no difference in the percentage of cartilaginous callus between treatment groups at any time point. Fracture union was achieved by 3 weeks in both *ia/ia* and control rats, again with no difference in the percentage of cartilaginous callus.

In contrast, marked differences in hard callus were evident in the ZA treated groups. ZA increased callus bone mineral content, volume and importantly increased callus strength. Bolus ZA treatment did not delay the commencement of hard callus remodelling at 4 weeks post fracture, whereas this was delayed in the Weekly ZA group. By 12 and 26 weeks Bolus ZA had the same callus content of remodelled neo-cortical bone as Saline, however Weekly ZA had significantly less than saline at these times. These extensive delays in hard callus remodelling with Weekly ZA dosing produced a fracture callus of inferior material properties.

In conclusion, neither ZA treatment nor the absence of active osteoclasts in *ia/ia* rats delayed endochondral fracture union. Thus, this study confirms the redundancy of osteoclasts in this process. Bolus ZA treatment was superior to Weekly ZA dosing; hard callus remodelling proceeded, producing a strong fracture callus with improved material properties. This study supports the use of less frequent ZA doses during fracture repair.