Mechanisms of Osteoarthritis: Interrelationships between Bone and Cartilage

A Thesis submitted to The University of Adelaide in fulfilment of the requirements for the degree of Doctor of Philosophy

By

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ABSTRACT

Osteoarthritis (OA) is a progressive joint disease and a common cause of disability. OA is characterised by loss of articular cartilage, subchondral bone sclerosis, cysts, and osteophyte formation. Increased subchondral bone remodelling plays an important role in the pathophysiology of OA and is associated with disease progression. It is known that Osteoprotegerin (OPG), receptor activator of nuclear factor kappa b (RANK) and its ligand RANKL tightly control bone remodelling. In addition, RANK, RANKL and OPG gene expression has been shown to be dysregulated in human OA subchondral bone.

Commonly OA is diagnosed at advanced stages, which makes it difficult to study the initiating events in the human disease. Animal models of OA are of considerable importance to study the progressive changes in OA, and to evaluate suitable OA drugs. Alendronate (ALN) is a potent bone resorption inhibitor and clinical trials using bisphosphonates to treat OA have yielded mixed results. This suggests that the effects of bisphosphonates may or may not be beneficial depending on the stage of OA progression.

The first aim of this thesis was to characterise the temporal structural changes of tibial articular cartilage and subchondral bone in a low-dose MIA-induced OA rat model. The results from micro-CT analysis showed that the tibiae of the MIA-injected knees had significant bone loss at 2 weeks (early OA), followed by increased bone volume, trabecular thickness and separation at 6 weeks (intermediate OA) and 10 weeks

(advanced OA). Micro-CT images revealed subchondral bone sclerosis, cysts, and osteophyte formation at 6 and 10 weeks. Histology revealed progressive cartilage degradation characteristic of the human disease.

The second aim of this thesis was to study the effect of ALN treatment initiated at day 0 (pre-emptive), week 2 (early treatment), and week 6 (delayed treatment) in a low-dose MIA rat model. To address the second aim the efficacy of ALN was tested on cartilage degradation, subchondral bone remodelling, and joint discomfort observed in this animal model. The study demonstrated that pre-emptive ALN treatment preserved subchondral trabecular bone microarchitecture, decreased bone turnover, prevented joint discomfort, and offered moderate chondroprotection. Early and delayed ALN treatment prevented loss of trabeculae and decreased bone turnover but did not have any identified effect on cartilage.

Finally, the RANK, RANKL, OPG gene expression in OA was characterised in a low-dose MIA rat model. The effect of ALN treatment on subchondral bone RANK, RANKL, and OPG gene expression at 2, 6, and 10 weeks after OA induction was assessed. This study showed that the RANKL and OPG gene expression was dysregulated in this animal model. In addition, the efficacy of ALN on early subchondral bone changes appears to occur through the modulation of RANKL and OPG gene expression.

Collectively, these findings demonstrate that the low-dose MIA rat model closely mimics the pathological features of progressive human OA disease. Moreover, this animal model showed a clear relationship between the cartilage damage and subchondral bone changes. ALN treatment preserved subchondral trabecular bone microarchitecture and decreased bone turnover. In addition, ALN prevented RANKL and OPG gene dysregulation in OA subchondral bone. Normalising subchondral bone remodelling offers an optimal treatment option and future drug intervention studies focusing on subchondral bone would provide improved treatment options for OA.

DECLARATION

I, Geetha Mohan certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference has been made in the text.

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Mohan G, Perilli E, Kuliwaba JS, Humphries JM, Parkinson IH, Fazzalari NL (2011) Application of *in vivo* micro-computed tomography in the temporal characterisation of subchondral bone architecture in a rat model of low-dose monosodium iodoacetate-induced osteoarthritis. *Arthritis Res Ther* 13:R210.

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Date

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PUBLISHED ABSTRACTS (reviewed)

Mohan G, Perilli E, Kuliwaba JS, Parkinson IH, Humphries JH, Fazzalari NL (2011) Characterisation of temporal subchondral bone changes in a rat model of low-dose monosodium iodoacetate induced osteoarthritis: An *in vivo* micro-CT study. *Osteoarthritis Cartilage* 19:S61-S62.

Mohan G, Perilli E, Kuliwaba JS, Parkinson IH, Humphries JH, Fazzalari NL (2011) Alendronate treatment reduces tibial subchondral bone damage in early-stage osteoarthritis: An *in vivo* micro-CT study in a rodent model. *Osteoporos Int* 22:S579.

SCIENTIFIC COMMUNICATION

Mohan G, Perilli E, Kuliwaba JS, Parkinson IH, Humphries JM, Fazzalari NL. Positive Effect of Alendronate on Tibial Subchondral Bone loss and Cartilage Repair at early stage experimental osteoarthritis in rats. *2011 Annual Scientific and Clinical Meeting & Annual General Meeting of the Australian Rheumatology Association (ARA) (South Australian Branch)*. Adelaide, Australia, October 28, 2011.

Mohan G, Perilli E, Kuliwaba JS, Parkinson IH, Humphries JM, Fazzalari NL. Characterisation of temporal subchondral bone changes in a rat model of low-dose monosodium iodoacetate induced osteoarthritis: An *in vivo* micro-CT study. *2011 OARSI world congress of Osteoarthritis Research Society International (OARSI).* San Diego, CA, USA, September 15 – 18, 2011.

Mohan G, Perilli E, Kuliwaba JS, **Parkinson IH,** Humphries JM, Fazzalari NL. Alendronate treatment reduces tibial subchondral bone damage at early-stage osteoarthritis: an *in vivo* micro-CT study in a rodent model. *The 21st Annual Scientific Meeting of the Australian and New Zealand Bone and Mineral Society (ANZBMS).* Gold Coast, Australia, September 4 – 8, 2011.

Mohan G, Perilli E, Kuliwaba JS, **Parkinson IH,** Humphries JM, Fazzalari NL. Tibial subchondral bone damage in early-stage osteoarthritis is reduced by Alendronate administration: an *in vivo* micro-CT study in a rodent model. *17th Annual Scientific Meeting of the Australian & New Zealand Orthopaedic Research Society (ANZORS).* Brisbane, Queensland, Australia, September 1 – 2, 2011.

Mohan G, Perilli E, Kuliwaba JS, Parkinson IH, Humphries JM, Fazzalari NL. Alendronate inhibits early subchondral bone loss and joint discomfort by modulating the RANKL/OPG nexus in a rodent model of osteoarthritis. *The University of Adelaide, Faculty of Health Sciences Postgraduate Research Conference.* Adelaide, Australia, August 25, 2011.

Mohan G, Perilli E, Kuliwaba JS, Parkinson IH, Humphries JM, Fazzalari NL. Anti-resorptive efficacy of alendronate treatment on tibial subchondral bone at early-stage osteoarthritis: an *in vivo* micro-CT study in a rodent model. *Annual scientific meeting of the Australian Society for Medical Research SA Scientific Meeting (ASMR) (South Australian Division).* Adelaide, Australia, June 8, 2011.

Mohan G, Perilli E, Kuliwaba JS, Humphries JM, Parkinson IH, Fazzalari NL. Application of *in vivo* micro-computed tomography in the characterisation of a rat model of osteoarthritis. *The 16th Annual Scientific Meeting of the ANZORS*. Melbourne, Australia, November 14 – 18, 2010.

Mohan G, Perilli E, Kuliwaba JS, Humphries JM, Parkinson IH, Fazzalari NL. Characterisation of a non-trauma rat model of iodoacetate induced osteoarthritis. *2010 Annual Scientific meeting of the ARA (South Australian Branch).* Adelaide, Australia, October 22, 2010.

Mohan G, Perilli E, Kuliwaba JS, Humphries JM, Parkinson IH, Fazzalari NL. *In vivo* imaging of subchondral bone using micro-CT in a rodent model of osteoarthritis. *The 20th Annual Scientific Meeting of the ANZBMS*. Adelaide, Australia, September 5 – 8, 2010.

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Mohan G, Perilli E, Kuliwaba JS, Humphries JM, Parkinson IH, Fazzalari NL. *In vivo* imaging of subchondral bone using micro computed tomography: A novel method to track longitudinal changes in a rodent model of osteoarthritis. Annual scientific meeting of the ASMR *(South Australian Division).* Adelaide, Australia, June 9, 2010.

Mohan G, Perilli E, Kuliwaba JS, Humphries JM, Parkinson IH, Fazzalari NL. Articular cartilage and subchondral bone changes in the monosodium iodoacetate rat model of osteoarthritis: A dose-response study. *The Sixth Clare Valley Bone Meeting.* Clare, Australia, March 26 – 30, 2010.

Mohan G, Perilli E, Kuliwaba JS, Parkinson IH, Fazzalari NL. Cartilage and subchondral bone changes in osteoarthritis: *In vivo* Micro-CT, Serological, and Histological Evaluation in a Rodent Model. *2009 Annual Scientific meeting of the ARA (South Australian Branch).* Adelaide, Australia, November 6, 2009.

Mohan G, Perilli E, Kuliwaba JS, Parkinson IH, Fazzalari NL. Cartilage and subchondral bone changes in osteoarthritis: *in vivo* micro-CT, serological and histological evaluation in a rodent. *15th Annual Scientific Meeting of the ANZORS.* Adelaide, Australia, October 8 – 10, 2009.

Mohan G, Perilli E, Kuliwaba JS, Parkinson IH, Fazzalari NL. Longitudinal subchondral bone changes in a rodent model of osteoarthritis using *in vivo* micro-computed tomography. *Annual scientific meeting of the ASMR (South Australian Division).* Adelaide, Australia, June 2, 2009.

AWARDS

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Best poster presentation award at The University of Adelaide, Faculty of Health Sciences Postgraduate Research Conference. Adelaide, Australia, August 25, 2011.

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ABBREVIATIONS

2D	Two dimensional
3D	Three dimensional
ACLT	Anterior cruciate ligament transection
ADAMTS	A disintegrin and metalloproteinase with thrombospondin motifs
ALN	Alendronate
BML	Bone marrow lesions
BMP	Bone morphogenetic proteins
BV	Bone volume
BV/TV	Bone volume fraction
COMP	Cartilage oligomeric matrix protein
CRP	C-reactive protein
CT	Cycle threshold
CTX-I	C-terminal telopeptide of collagen type I
CTX-II	C-terminal telopeptide of collagen type II
DEXA	Dual energy x-ray absorptiometry
Dkk-1	Dickkopf-1
FE	Finite element
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
HLWB	Hind limb weight-bearing
IL-1	Interleukin-1
KVp	Kilovoltage peak
MAPK	Mitogen activated protein kinase

MAR	Mineral apposition rate
MIA	Monosodium iodoacetate
Micro-CT	Micro computed tomography
ml	Millilitres
mm	Millimetre
MMPs	Metalloproteinases
MRI	Magnetic resonance imaging
NFAT	Nuclear factor of activated T cells,
NTX	N-terminal type I collagen telopeptides
OA	Osteoarthritis
OARSI	Osteoarthritis Research Society International
ºC	degrees Celsius
OPG	Osteoprotegerin
OVX	Ovariectomized
Pl.Por	Plate porosity
Pl.Th	Plate thickness
RANK	Receptor activator of nuclear factor κB
RANKL	Receptor activator of nuclear factor κB ligand
ROI	Region of interest
RT	Reverse transcription
SPECT	Single-photon–emission computed tomography
Tb.N	Trabecular number
Tb.Sp	Trabecular separation
Tb.Th	Trabecular thickness

TGF-β	Transforming growth factor-β
TNFα	Tumor necrosis factor alpha
TRAF	TNFR-associated factor proteins
TRAP	Tartrate-resistant acid phosphatase
vBMD	Volumetric bone mineral density
VEGF	Vascular endothelial growth factor
VOI	Volume of interest
Wnt	Wingless-type MMTV integration site
μA	Microampere
μl	Micro litres
μm	Micrometres