THE EFFECTS OF GLYCERYL TRINITRATE AND OVARIECTOMY ON FEMORO-TIBIAL ARTICULAR CARTILAGE, SYNOVIUM AND SUBCHONDRAL BONE IN NORMAL AND OSTEOARTHRITIC EWES

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This thesis is presented for the degree of Doctor of Philosophy of Murdoch University

2002
I declare that this thesis is my own account of my research, and contains as its main content work which has not previously been submitted for degree at any tertiary educational institution.

Martin Cake
8th February 2002
ABSTRACT

Nitric oxide (NO) alters chondrocyte metabolism, and is thought to be a key catabolic mediator in osteoarthritis. NO is also an important modifier of bone metabolism, and may partially mediate the bone-sparing effects of oestrogen. Oestrogen has also been linked to the modulation of osteoarthritis, though its role is not clear. The aim of this study was to examine the structural and metabolic effects of ovariectomy and the NO donor glyceryl trinitrate (GTN) on (1) normal ovine femoro-tibial joint tissues, and (2) the progression of joint lesions in the established ovine meniscectomy model of osteoarthritis.

Preliminary investigations tested a novel computer-assisted histomorphometric method of assessing osteochondral changes post-meniscectomy, in a trial of a putative disease-modifying osteoarthritis compound. Quantitative assessment revealed a subtle protective effect not evident by qualitative methods. These techniques were then used to test the experimental hypotheses in a combined trial involving 48 aged ewes, variously subjected to ovariectomy, bilateral lateral meniscectomy, and/or topical GTN therapy. At six months, joint tissues were analysed using histology, histomorphometry, dynamic biomechanical testing, serum markers, bone densitometry, and tissue culture of synovial fibroblasts and explants of cartilage and bone. Ovariectomy modified cartilage structure and chondrocyte metabolism, and induced subchondral bone remodelling. Prior ovariectomy altered the development of OA lesions post-meniscectomy, producing thicker but biomechanically inferior cartilage and elevated metabolic activity in subchondral bone. GTN treatment of normal sheep induced thinner, structurally-altered cartilage in normal sheep, and accentuated cartilage and subchondral bone lesions post-meniscectomy. These results support an important homeostatic role for oestrogen in joint tissues, and show that GTN, a commonly used angina therapy, can induce structural alterations in joint tissues and potentially accelerate the progression of concurrent OA. Results also advance understanding of the role of synovial and subchondral bone changes in the pathogenesis of this OA model.
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PUBLICATIONS AND CONFERENCE PRESENTATIONS RESULTING FROM THIS WORK

* updated for this electronic version of thesis Dec 2005

REFEREED JOURNAL ARTICLES


Cake MA, Appleyard RC, Read RA, Smith MM, Murrell GAC, Ghosh P (2005) Ovariectomy alters the structural and biomechanical properties of ovine femoro-tibial articular cartilage and increases cartilage iNOS. *Osteoarthritis and Cartilage* 13(12):1066-1075

PRESENTATIONS AT SCIENTIFIC MEETINGS


ACKNOWLEDGEMENTS

I would like to sincerely thank the following people for their assistance with this study:

Associate Professors Rick Read (Murdoch University) and Peter Ghosh (University of Sydney) for their constant guidance and inspiration.

Mrs Diana Pethick, David Brockway, Peter Clune, Jim Poynton, and other staff of the Division of Veterinary and Biological Sciences, Murdoch University, for their tireless assistance with all animal procedures.

All staff of Raymond Purves Bone and Joint Research Laboratories, Institute of Bone and Joint Research (Royal North Shore Hospital, Sydney) for their friendship, stimulating discussion, and helpful suggestions. In particular, thanks to Ms Susan Smith for her competent preparation of the histological sections, and Hwa Su-Yang for his help with DEXA measurements.

Ms Joanna Makowey and Prof. Philip Sambrook of the Department of Rheumatology, Royal North Shore Hospital for the use of the Hologic QDR 4500W densitometer.

Richard Appleyard and George Murrell of the Orthopaedic Research Institute, St George Hospital (University of NSW) for performing biomechanical testing and assisting with birefringence studies.

Dr Els Meusen and Garry Barcham of the Centre for Animal Biotechnology, University of Melbourne for kindly providing the ovine recombinant IL-113.

The Walter and Eliza Hall Institute, for kindly providing 7TD1 hybridoma cells.

Graeme Worth and Bob Retallack of the Endocrinology laboratory, Sir Charles Gairdner Hospital, for performing the osteocalcin assays.

Mark Walters and Roger Price (Sir Charles Gairdner Hospital) for their help with densitometry of lumbar vertebrae, and helpful discussions.

Graeme Martin and Margaret Blackberry of Animal Sciences, University of Western Australia for the use of their laboratories and assistance with plasma oestradiol assays.

I would especially like to thank the Murdoch University Veterinary Trust for providing my research scholarship, and Boehringer Ingelheim Vetmedica for additional financial support.

Finally, to Jen, Josie, Ellie and family, for all their love and support.
ABBREVIATIONS

ACL Anterior cruciate ligament
ACLT Anterior cruciate ligament transection
ACTH Adrenocorticotropic hormone
ADAM A disintegrin and metalloprotease
ADAM-TS A disintegrin and metalloprotease with thrombospondin motifs
ANOVA Analysis of variance
APMA p-aminophenylmercuric acetate
ASU Avocado-soya unsaponifiables
bFGF Basic fibroblast growth factor
BMD Bone mineral density
BMI Body mass index
CAF Chondrocyte activating factors
CM Conditioned medium/media
cNOS Constitutive (Ca\textsuperscript{2+}-dependant) nitric oxide synthase
COX Cyclooxygenase
DEXA Dual-energy x-ray absorptiometry
DMOADs Disease-modifying osteoarthritis drugs
dpi Dots per inch
DPyr Deoxypyridinoline
EDRF Endothelial-derived relaxation factor
EDTA Ethylenediaminetetraacetic acid
EGF Epidermal growth factor
eNOS Endothelial nitric oxide synthase
ER Estrogen (oestrogen) receptor
FGF Fibroblast growth factor
FSH Follicle-stimulating hormone
G* Corrected dynamic shear modulus
GTN Glycerol trinitrate
HA Hyaluronic acid/ hyaluronan
HEPES N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]
HPLC High-performance liquid chromatography
HRT Hormone replacement therapy
ICAM Intercellular adhesion molecule
ICE Interleukin-1 converting enzyme
IGF Insulin-like growth factor
IGFBP Insulin-like growth factor binding protein
IL Interleukin
IL-1Ra Interleukin-1 receptor antagonist
iNOS Inducible nitric oxide synthase
kD KiloDaltons
LFC Lateral femoral condyle
LH Luteinising hormone
LIF Leukocyte inhibitory factor
l-NAME N\textsuperscript{c}-nitro-L-arginine methylester (NOSI)
l-NIL L-N\textsuperscript{5}-iminoethyl-L-lysine (NOSI)
l-NMA L-N\textsuperscript{5}-monomethylarginine (NOSI)
LTP Lateral tibial plateau
MCM Monocyte-conditioned medium
<table>
<thead>
<tr>
<th>Acronym</th>
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<tr>
<td>MFC</td>
<td>Medial femoral condyle</td>
</tr>
<tr>
<td>MHC</td>
<td>Major histocompatibility complex</td>
</tr>
<tr>
<td>MMA</td>
<td>Methyl methacrylate</td>
</tr>
<tr>
<td>MMP</td>
<td>Makix metalloprotease</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>MT-MMP</td>
<td>Membrane-type matrix metalloprotease</td>
</tr>
<tr>
<td>MTP</td>
<td>Medial tibial plateau</td>
</tr>
<tr>
<td>MX</td>
<td>Meniscectomised</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>NOC</td>
<td>Non-operated control</td>
</tr>
<tr>
<td>NOS</td>
<td>Nitric oxide synthase</td>
</tr>
<tr>
<td>NOSI</td>
<td>Nitric oxide synthase inhibitor</td>
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<tr>
<td>NSAIDs</td>
<td>Non-steroidal anti-inflammatory drugs</td>
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<tr>
<td>OA</td>
<td>Osteoarthritis</td>
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<tr>
<td>OPG</td>
<td>Osteoprotegerin</td>
</tr>
<tr>
<td>OVX</td>
<td>Ovariectomised</td>
</tr>
<tr>
<td>PBM</td>
<td>Peripheral blood mononuclear cells</td>
</tr>
<tr>
<td>PDGF</td>
<td>Platelet-derived growth factor</td>
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<tr>
<td>PGE₂</td>
<td>Prostaglandin E₂</td>
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<tr>
<td>PLSD</td>
<td>Protected least significant difference</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate-buffered saline</td>
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<tr>
<td>PS-GAG</td>
<td>Polysulphated glycosaminoglycan</td>
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<tr>
<td>PPS</td>
<td>Pentosan polysulphate</td>
</tr>
<tr>
<td>Pyr</td>
<td>Pyridinoline</td>
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<tr>
<td>RA</td>
<td>Rheumatoid arthritis</td>
</tr>
<tr>
<td>RANKL</td>
<td>Receptor activator of NF-κB ligand</td>
</tr>
<tr>
<td>RIA</td>
<td>Radio-immunoassay</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
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<tr>
<td>rpm</td>
<td>Revolutions per minute</td>
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<tr>
<td>SCID</td>
<td>Severe combined immunodeficiency</td>
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<td>SCP</td>
<td>Subchondral bone plate</td>
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<td>SNAP</td>
<td>s-nitrosyl-n-acetylpenicillamine (organic NO donor)</td>
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<tr>
<td>TACE</td>
<td>TNF-α converting enzyme</td>
</tr>
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<td>TG</td>
<td>Trochlear groove</td>
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<td>Transforming growth factor-β</td>
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<tr>
<td>TIMP</td>
<td>Tissue inhibitor of matrix metalloproteases</td>
</tr>
<tr>
<td>tPA</td>
<td>Tissue-type plasminogen activator</td>
</tr>
<tr>
<td>UDPGD</td>
<td>Uridine diphosphoglucose dehydrogenase</td>
</tr>
<tr>
<td>UCC</td>
<td>Uncalcified cartilage</td>
</tr>
<tr>
<td>uPA</td>
<td>Urokinase-type plasminogen activator</td>
</tr>
<tr>
<td>uPAR</td>
<td>Urokinase-type plasminogen activator receptor</td>
</tr>
<tr>
<td>VCAM</td>
<td>Vascular cell adhesion molecule</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
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</table>
This thesis details the findings of sheep studies conducted between 1998 and 2002. Predominantly, these studies investigate the effects of ovariectomy, and the exogenous nitric oxide donor glycercyl trinitrate, on the structural and metabolic integrity of joint tissues in normal and osteoarthritic (meniscectomised) sheep. A large number of techniques were used to fully investigate the effects of these interventions, encompassing histology, biomechanical testing, bone densitometry, serum markers, and extensive tissue culture. Most of these investigations were conducted by the author, the most notable exception being the dynamic biomechanical testing of cartilage, which was carried out in conjunction with Dr. Richard Appleyard of St. George Hospital, Sydney.

Due to the length and scope of these studies, efficient use of time and subjects required that multiple hypotheses were tested within the design of a single large trial. For this reason, this thesis is composed of only four chapters: (Chapter 1) an introductory literature review and statement of hypotheses; (Chapter 2) a preliminary trial applying computerised image analysis methods to assess osteoarthritic changes in this sheep model; (Chapter 3) an extensive trial testing the main experimental hypotheses; and (Chapter 4) summary and conclusions. The testing of several hypotheses simultaneously within a single large trial may make it difficult for the reader to follow individual hypotheses through Chapter 3. It is important to note that the methods and results (3.2 and 3.3) relating to multiple hypotheses (stated in 1.3) are presented together (i.e. all eight experimental groups), before discussing each hypothesis individually in 3.4.

Reference is made within the text (and Appendix 1) to findings from another trial involving ovariectomised ewes, conducted simultaneously by our laboratories. Some of these findings have been presented in preliminary abstract form and are referenced as published work (i.e. Smith et al. (2000), Parker et al. (2000), Hwa et al. (2001)). Some of the findings of the present study are therefore described as confirmatory of these published works, but the reader should note these studies were essentially conducted in parallel.