The Effect of Recombinant Human Osteogenic Protein-1 on Growth Plate Repair in a Sheep Model

by

Belinda Jane Thomas

B.Sc. (Hons)

Department of Paediatrics
The University of Adelaide
Adelaide, South Australia

Department of Orthopaedic Surgery
Women's and Children's Hospital
Adelaide, South Australia

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Thesis Summary

The growth plate is a unique cartilaginous structure located towards the ends of children's long bones. It is responsible for the longitudinal growth of the bones through the proliferation, maturation and hypertrophy of chondrocytes, the specialised cells of the growth plate. The growth plate has a limited ability to undergo repair, and damage to it often results in limb angulation deformities and/or growth arrest due to the formation of a bone bridge spanning the growth plate region. The Langenskiöld procedure is a surgical treatment for growth plate injuries that involves the removal of the bone bridge and replacement with autologous fat. This prevents further bone formation and allows future growth of the bone.

Osteogenic protein-1 (OP-1) is a member of the transforming growth factor beta (TGF-β) superfamily and is well known for its ability to promote bone formation in diaphyseal defects. However, more recent research has demonstrated that OP-1 can also promote the proliferation of chondrocytes and the synthesis of a cartilage matrix both *in vitro* and *in vivo*. This growth factor has been successfully used to enhance healing of articular cartilage and thyroid defects *in vivo*.

The aim of this study was to further investigate the histological and molecular changes that occur to the remaining growth plate and defect site following the Langenskiöld procedure in the presence and absence of recombinant human OP-1 (rhOP-1). A sheep model was utilised in which a section of the growth plate was ablated and filled with autologous fat. Half of the animals had rhOP-1 (350 μg) injected at the interface of the remaining growth

plate and the defect. The animals were sacrificed in triplicate at days 7, 14 and 56, and the tissues processed for histological and molecular characterisation.

Following the Langenskiöld procedure, the total limb growth continued at an equivalent rate in both the rhOP-1 treated and untreated groups compared to the normal animals that did not undergo the Langenskiöld procedure. There were no differences in the formation of bone, medial spurs or fibrocartilage observed between the rhOP-1 treated and untreated groups. However, the rhOP-1 treated animals displayed limited fibrous capsule formation around the fat implant compared to the untreated animals. The animals that were treated with rhOP-1 did show a significant increase in the height of the growth plate adjacent to the defect compared to the height at the most distal aspect. The majority of the growth plate height increase was observed within the resting zone, and as there was no significant change in the number of cells present within the area, this was attributed to an increase in extracellular matrix synthesis by the resting chondrocytes.

Immunohistochemical analysis demonstrated that the growth plate adjacent to the defect displayed molecules consistent with the cartilage phenotype, including collagen types II and X, biglycan and glycosaminoglycan epitopes from chondroitin sulphate, chondroitin-4-sulphate and keratan sulphate. The presence of these molecules in both groups suggests that rhOP-1 does not have an adverse effect on molecules indicative of the chondrogenic phenotype. However, the expression of type I collagen, osteopontin and decorin was detected in the chondrocytes adjacent to the defect in the rhOP-1 treated animals at day 7 and the untreated animals at day 56. These molecules are usually indicative of an osteogenic phenotype and suggest a modulation of chondrocyte phenotype within the growth plate. These molecules were detected in both the rhOP-1 treated and untreated

groups suggesting that the phenotypic switch was not a direct result of the rhOP-1 treatment. Rather, treatment with rhOP-1 accelerated the response, with the molecules appearing at day 7 compared to day 56.

In conclusion, administration of rhOP-1 in conjunction with the Langenskiöld procedure initiated a complex response in the growth plate adjacent to the defect. There was a significant increase in growth plate height, suggesting this growth factor may be beneficial in regenerating the growth plate following injury. However, rhOP-1 also accelerated the osteogenic response that was observed in the untreated animals. Therefore, the use of rhOP-1 in the treatment of growth plate injuries may be of limited value. The osteogenic properties of this growth factor have the potential to cause accelerated bone formation due to the osteogenic phenotype the growth plate chondrocytes adopted.

Declaration

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, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Belinda Jane Thomas

February 2004

This thesis is dedicated to the memory of

David Roy Thomas,

Frederick George Moll

and

Amelia Lousie Rix

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Abbreviations

APES	3-aminopropyltriethoxysilane
bp, kb	base pairs, kilo base pairs
BSA	bovine serum albumin
°C	degrees Celsius
dH_2O	deionised water
DIG	digoxigenin
DNA	deoxyribonucleic acid
EDTA	ethylene diamine tetra acetic acid
ELISA	enzyme linked immunosorbant assay
g, mg, µg, ng	grams, milligrams, micrograms, nanograms
K-wires	Kirschner wires
L, ml, µl	litre, millilitre, microlitre
MGP	matrix γ-carboxyglutamic acid protein
mm, µm	millimetre, micrometre
M, mM	moles per litre, millimoles per litre
µCi	micro Curie
OD	optical density
opm	oscillations per minute
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
pH	hydrogen ion concentration
% (w/v)	percent weight per volume
% (v/v)	percent volume per volume
rhOP-1	recombinant human osteogenic protein-1
RNA	ribonucleic acid
rpm	revolutions per minute
SDS	sodium dedecyl sulphate
SSC	saline sodium citrate
Tris	Tris (hydroxymethyl) amino methane
U	units
UV	ultra violet
V	volts

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