The dose related effects of phenylbutazone and a methylprednisolone acetate formulation (Depo-Medrol®) on cultured explants of equine carpal articular cartilage.

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ABSTRACT

Experimental methods involving the maintenance of explants of equine articular cartilage in tissue culture, an amino sugar assay, radiolabelling, and histology were developed and validated.

The dose related effects of phenylbutazone and Depo-Medrol® on chondrocyte viability and chondrocyte mediated synthesis and depletion of proteoglycans were investigated using cultured explants of equine middle carpal joint articular cartilage. Explants from 12 horses (941 x 3 mm diameter) were cultured for a total of 5 days, which included 3 days exposure to either phenylbutazone (0, 2, 20, 200, 2000 μg mL⁻¹), or Depo-Medrol (0, 20, 200, or 2000 μg mL⁻¹). For each explant, amino sugar content was used as a measure of proteoglycan content, ³⁵S incorporation as a measure of the rate of proteoglycan synthesis, and the number of pyknotic nuclei as a measure of cell death.

During culture, control explants remained metabolically active and viable but suffered a net loss of proteoglycans. Proteoglycan loss was reduced by the presence of either phenylbutazone or Depo-Medrol. This effect was significant at clinically relevant concentrations of phenylbutazone (2-20 μg mL⁻¹), but not Depo-Medrol (20-200 μg mL⁻¹). Depo-Medrol caused a dose-dependent suppression of proteoglycan synthesis at all concentrations, but chondrocyte viability was affected at only the 2000 μg mL⁻¹ dose. Phenylbutazone affected proteoglycan synthesis and cell viability at only the 2000 μg mL⁻¹ concentration. At all concentrations, the anti-catabolic effects of each drug influenced the proteoglycan content of the explants far more than did any anti-anabolic or cytotoxic drug effect.

The results suggest that the therapeutic potential of both phenylbutazone and Depo-Medrol may not be just restricted to their anti-inflammatory effects on the soft tissues of the joint, but may also involve a suppression of the synthesis and/or activation of proteolytic enzymes within the cartilage itself.
Lameness has been reported as the number one cause of lost training days and failure to race in the thoroughbred industry (Jeffcott et al., 1982; Rossdale et al., 1985). Joint associated lameness accounted for a third of the lamenesses localised. Causes of joint lameness include soft tissue inflammations, infections, osteochondritis disicans, degenerative joint disease, ligamental problems and intra-articular fractures. All of the above conditions may progress to degenerative joint disease.

Corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to treat degenerative joint disease (osteoarthritis) in animals and man. Degenerative joint disease is characterised by deterioration of the articular cartilage, accompanied by changes to the bone and soft tissues of the joint (McIlwraith, 1982). Treatment aims include resolution of initiating causes, restoration of function, and prevention of further articular cartilage damage (McIlwraith & Vachon, 1988). Because the reparative response of articular cartilage is inadequate (Desjardins & Hurtig, 1990), loss of articular cartilage often limits the complete restoration of athletic function (Bramlage et al., 1988; Richardson & Clark, 1991).

The pathogenesis of degenerative joint disease is incompletely understood (McIlwraith & Vachon, 1988). However, the release of proteoglycans is recognised as one of the earliest responses of articular cartilage to injury (Mankin, 1974; Clyne, 1987). It has been proposed that proteoglycan depletion resulting from increased proteoglycan catabolism may leave the chondrocytes and the collagen structural framework more susceptible to further mechanical damage and thus perpetuate the cycle of degeneration (Harris et al., 1972; McIlwraith & Van Sickle, 1981). The relative significance of chondrocyte mediated proteoglycan catabolism versus that mediated by enzymes released from the synoviocytes and migrant leucocytes has not been established (Fell & Jubb, 1977; McIlwraith & Van Sickle, 1981; Martel-Pelletier et al., 1984; Hurtig, 1988; McIlwraith & Vachon, 1988; May et al., 1991). Cytokine and drug induced
suppression of proteoglycan synthesis may also contribute to the proteoglycan depletion in some osteoarthritic conditions (Palmoski & Brandt, 1983; MacDonald et al., 1992; May et al., 1992).

Methylprednisolone acetate (MPA) and phenylbutazone (PBZ) are the most common steroid and non-steroidal anti-inflammatory drugs used for treatment of joint injury in equine athletes. Their soft tissue mediated clinical effects are well recognised (Higgins & Lees, 1984). Whether or not they also confer some degree of chondroprotection is actively debated (Tobin et al., 1986; Burkhardt & Ghosh, 1987; McIlwraith, 1989). Furthermore, there is some evidence to suggest their use may actually potentiate the progression of joint deterioration (Whitehouse & Bostrum, 1962; Tobin et al., 1986; Chunekamrai et al., 1989; Trotter et al., 1991; Shoemaker et al., 1992). Both the types and mechanisms of their effects on articular cartilage are subjects of some conjecture and much controversy (May et al., 1987; McIlwraith & Vachon, 1988; Saari et al., 1992).

Relatively few controlled in vivo trials have sought to investigate the effects of MPA or PBZ on equine articular cartilage. Interpretation of specific drug effects from these trials has been hindered by their small sample numbers, the types of investigative procedures performed, and a range of confounding variables. The in vitro maintenance of tissue allows for a more controlled environment in which the study of specific interactions can be isolated from confounding variables (Tyler et al., 1982).

The purpose of this study was to investigate the dose related effects of phenylbutazone, and a methylprednisolone acetate formulation (Depo-Medrol®), on chondrocyte viability, and chondrocyte mediated degradation and synthesis of matrix proteoglycans so as to better understand how these drugs exert their effects in vivo. The following three hypotheses were tested with respect to each of these parameters; (1) the drug is capable of affecting the parameter, (2) the effect is apparent at clinically relevant concentrations, and (3) the effect is greater at higher concentrations.

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>PREFACE</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xiii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xviii</td>
</tr>
</tbody>
</table>
INTRODUCTION

A. Articular cartilage structure and function

1. The chondrocytes ............................................. 2
2. Collagen 4
3. The proteoglycans ............................................. 5
4. Measures of matrix biosynthesis and depletion .......... 7
5. Response to injury ............................................. 11

B. Use of intra-articular corticosteroids in the horse

1. Anti-inflammatory mechanism of action ..................... 14
2. Manufacturer recommendations ............................... 18
3. Pharmacokinetics ............................................. 19
4. Clinical use .................................................... 21
5. Adverse effects on joints .................................... 22

C. Effects of corticosteroids on articular cartilage

1. Normal articular cartilage ................................... 24
   (i) Chondrocyte morphology ................................. 25
   (ii) Matrix biosynthesis and depletion;
        in vivo results ........................................ 25
   (iii) Matrix biosynthesis and depletion;
        in vitro results ...................................... 27
2. Articular cartilage of arthritic joints ..................... 28
3. Articular cartilage repair .................................. 29
D. Use of phenylbutazone in the horse

1. Molecular mechanism of action ........................................... 31
2. Manufacturer recommendations ......................................... 34
3. Pharmacokinetics
   (i) Absorption ................................................................. 35
   (ii) Distribution .............................................................. 35
   (iii) Metabolism and elimination ....................................... 37
   (iv) Oxyphenbutazone kinetics ......................................... 38
4. Clinical use ................................................................. 39
5. Adverse effects on joints .................................................. 40

E. Effect of phenylbutazone on articular cartilage

................................................................. 40

F. Summary

................................................................. 42

G. Objectives

................................................................. 43

H. Hypotheses

................................................................. 43
MATERIALS AND METHODS

SECTION I: DEVELOPMENT OF THE MODEL

A. Analytical techniques

1. Biochemical analyses ........................................ 44
2. Scintillation counting ........................................ 54
3. DNA assay .................................................... 64

B. Measurement of proteoglycans in the culture media

1. Factors affecting the amino sugar assay ..................... 67
2. Methods to reduce media and drug interference ............. 72
3. Separation of chondroitin sulphate from solutions
   containing serum proteins .................................... 79
4. Separation of proteoglycans from solutions containing
   serum proteins ................................................ 89
5. Summary ....................................................... 93

C. Explant culture characteristics

1. Variation in the amino sugar content and rate of $^{35}$S
   incorporation between explants from different sites .......... 96
2. Viability of chondrocytes in cultured explants of
   equine articular cartilage ................................... 99
3. Variation in the biosynthetic rate and total amino sugar
   concentration relative to time cultured ...................... 106
SECTION II: USE OF THE MODEL

A. Explant Culture

1. Source of articular cartilage ........................................ 111
2. Preparation of cartilage samples ................................. 111
3. Methylprednisolone acetate trial ............................... 114
4. Phenylbutazone trial .................................................. 115

B. Post-culture processing

1. Explants from the radial carpal bones ......................... 115
2. Explants from the third carpal bones ......................... 116

C. Analytical techniques

1. Amino sugar assay ....................................................... 116
2. Scintillation counting procedure ................................. 116
3. Histological evaluation .............................................. 117

D. Statistical methods

1. Explants from the radial carpal bones ......................... 117
2. Explants from the third carpal bones ......................... 117
RESULTS

A. Depo-Medrol (MPA) trial

1. Effect of Depo-Medrol on amino sugar content .......................... 119
2. Effect of Depo-Medrol on $^{35}$S incorporation .......................... 119
3. Effect of Depo-Medrol on chondrocyte viability .......................... 122

B. Phenylbutazone (PBZ) trial

1. Effect of PBZ on amino sugar content ....................................... 124
2. Effect of PBZ on $^{35}$S incorporation ....................................... 124
3. Effect of PBZ on chondrocyte viability ..................................... 127

C. Histological trial observations

.......................................................... 129
DISCUSSION

A. Discussion of the methods .................................................. 134

B. Discussion of the results ...................................................... 140

C. Summary and conclusions .................................................... 148

APPENDIX

A. Tables ................................................................................. 150

B. Statistical analyses .............................................................. 161

BIBLIOGRAPHY ........................................................................ 170
FIGURES

2.1 Comparison of the absorption spectra of glucosamine and galactosamine (from 5-20 μg) ........................................... 47

2.2 Comparison of the absorption spectra of equivalent amounts of glucosamine, galactosamine, and chondroitin sulphate .......... 47

2.3 Absorption spectra of digested cartilage ................................................. 49

2.4 Comparison of the standard curves of glucosamine, galactosamine, and chondroitin sulphate ........................................... 50

2.5 The gradation of colours produced by a range of glucosamine amounts (2.5-20 μg) after being assayed according to the method of Gatt & Berman. The cuvette containing the yellow/orange solution is an example of the sensitivity of the assay to any variation in acidity ................................................................. 51

2.6 Stability of the amino sugar assay over time ........................................... 53

2.7 Custom made cartilage chisel ................................................................. 55

2.8 Cartilage punches ................................................................. 56

2.9 Quench curve generated by 14C quenched standards ........................... 60

2.10 The effect of different wash procedures on the non-bound 35S content of explants of equine articular cartilage .......................... 62

2.11 Standard curve for calf thymus DNA assayed according to the method of Labarca and Paigen (1980) .......................... 66
2.12 Absorption spectra of glucosamine standards dissolved in water ... 68

2.13 Absorption spectra of glucosamine standards dissolved in DMEM ... 68

2.14 Absorption spectra of glucosamine standards dissolved in DMEM
and assayed after oven digestion in 2N HCl .......................... 68

2.15 Absorption spectra produced by DMEM, aqueous phenol red,
and a 25% aqueous DMEM solution in response to the Gatt &
Berman (1966) amino sugar assay (relative to a H2O blank,
A = 0.5) ................................................................. 74

2.16 Absorption spectra of chondroitin sulphate standards dissolved
in DMEM following dialysis and oven digestion in 2N HCl
(A = 2.0) ................................................................. 74

2.17 Absorption spectra of DMEM and gentamicin following dialysis
and oven digestion in 2N HCl (relative to a H2O blank,
A = 2.0) ................................................................. 77

2.18 Comparison of chondroitin sulphate standards dissolved in water
and DMEM after dialysis and oven digestion ...................... 78

2.19 The absorption spectrum produced by a crude papain solution .... 81

2.20 Comparison of the absorption spectrum of glucosamine, and a
glucosamine + papain combination .................................... 83

2.21 Effect of papain on the assay of glucosamine standards ............ 84

2.22 Effect of serum protein precipitation on the amino sugar
concentration of chondroitin sulphate standards .................... 87
2.23 Effect of serum protein precipitation and dialysis on the absorption spectra of aqueous chondroitin sulphate standards .... 88

2.24 Effect of serum protein precipitation and dialysis on the absorption spectra of chondroitin sulphate standards dissolved in DMEM .................................................. 88

2.25 Effect of culture duration on the viability of chondrocytes within explants of equine articular cartilage ...................... 102

2.26 Photomicrograph showing cell death at the edge of a cultured explant of equine carpal articular cartilage .................. 103

2.27 Photomicrograph showing cell death at the articular edge of a cultured explant of equine carpal articular cartilage .......... 104

2.28 Photomicrograph showing cell vacuolation in a cultured explant of equine carpal articular cartilage ....................... 105

2.29 Effect of culture duration on the amino sugar content of explants relative to their initial wet weight ....................... 108

2.30 Effect of culture duration on the amino sugar content of explants relative to their final DNA content ...................... 108

2.31 Effect of culture duration on $^{35}$S incorporation by explants relative to their initial wet weight ............................ 109

2.32 Effect of culture duration on $^{35}$S incorporation by explants relative to their final DNA content ............................ 109
3.1 Photograph of an open equine middle carpal joint showing the sites (top = third carpal bone, bottom = radial carpal bone) where the strips of articular cartilage were harvested from ... 112

3.2 Strip of articular cartilage from which explants have been cut ... 113

3.3 Explants suspended in chilled DMEM awaiting further processing ... 113

4.1 Effect of Depo-Medrol on the amino sugar content of cultured explants of equine middle carpal joint articular cartilage ... 120

4.2 Effect of Depo-Medrol on $^{35}$S incorporation by cultured explants of equine middle carpal joint articular cartilage ... 121

4.3 Effect of Depo-Medrol on the viability of chondrocytes within cultured explants of equine middle carpal joint articular cartilage ... 123

4.4 Effect of phenylbutazone on the amino sugar content of cultured explants of equine middle carpal joint articular cartilage ... 125

4.5 Effect of phenylbutazone on $^{35}$S incorporation by cultured explants of equine middle carpal joint articular cartilage ... 126

4.6 Effect of phenylbutazone on the viability of chondrocytes within cultured explants of equine middle carpal joint articular cartilage ... 128

4.7 Photomicrograph of an empty lacunae with the displaced nucleus sitting adjacent (1000x magnification) ... 130
4.8 Photomicrograph of two dead chondrocytes close to the articular surface (4000x magnification) ........................................ 131

4.9 Photomicrograph of a chondrocyte cluster
(4000x magnification) ..................................................... 132

4.10 Comparison of the variation in proteoglycan content, as shown by alcian blue staining, of two sections cut from non-cultured explants harvested from the radial carpal bone of the same horse (400x magnification) ........................................ 133
### TABLES

2.1 DNA assay of digested cartilage samples .................................. 65

2.2 Chemical composition of Dulbecco’s modified Eagle medium (DMEM) ............................................................... 70

2.3 Amino sugar assay absorbances following the addition of TCA and dialysis (524 nm) ................................................ 90

2.4 Amino sugar assay absorbances following the addition of NH$_4$SO$_4$ and dialysis (524 nm) ....................................... 93

2.5 Variability in the amino sugar content (AS) of cultured explants from different carpal bones (µg mg$^{-1}$) ..................... 98

2.6 Variability in the incorporation of $^{35}$S by cultured explants from different carpal bones (dpm mg$^{-1}$) ....................... 98

2.7 Chondrocyte death relative to time cultured (dead/total) .......... 101