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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 $\mu g\ mL^{-1}$), or Depo-Medrol (0, 20, 200, or 2000 $\mu g\ mL^{-1}$). For each explant, amino sugar content was used as a measure of proteoglycan content, ^{35}S 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 $\mu g\ mL^{-1}$), but not Depo-Medrol (20-200 $\mu g\ mL^{-1}$). Depo-Medrol caused a dose-dependent suppression of proteoglycan synthesis at all concentrations, but chondrocyte viability was affected at only the 2000 $\mu g\ mL^{-1}$ dose. Phenylbutazone affected proteoglycan synthesis and cell viability at only the 2000 $\mu g\ mL^{-1}$ 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.

PREFACE

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®)[1], 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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[1] Depo-Medrol, Upjohn Inter-American Corporation.

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