Dexamethasone Partially Inhibits the Deleterious Effects of Lipopolysaccharides on the Metabolism of Chondrocytes Cultured in Alginate Beads

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INTRODUCTION: Articular cartilage exposed to lipopolysaccharides (LPS) molecules undergoes rapid depletion of proteoglycans (PGs), both in vitro and in vivo [1,2]. LPS is one of the major factors playing a role in the pathogenesis of septic arthritis and Lyme arthritis [3]. Dexamethasone, member of the glucocorticoid class of steroid hormones, acts as an anti-inflammatory and immunosuppressant. Some studies have shown that dexamethasone inhibits PG synthesis and also suppresses the expression of the genes for aggrecan and collagen type II [4]. It also was reported that dexamethasone suppresses the gene expression and activity of matrix metalloproteinases (MMP-1, 3, and 13) [4]. Taken together these findings suggest that dexamethasone reduces the metabolic activity of a chondrocyte – both its synthesis and turnover. On the other hand, studies have also shown that dexamethasone can promote proteoglycan synthesis in rabbit costal chondrocyte cultures and human articular chondrocytes [5,6]. Thus the purpose of this study was (i) to investigate the effect of dexamethasone on the metabolism of chondrocytes cultured in alginate beads, and (ii) to investigate whether the effect of dexamethasone on the metabolism of chondrocytes can be used to protect the chondrocytes from the deleterious effects of LPS.

METHODS: Bovine articular chondrocytes were isolated and suspended in 1.2% alginate beads at 4 million cells/ml. The beads were pre-cultured for 7 days in complete medium (DMEM/F12 supplemented with 360 µg/ml L-glutamate and 25 µg/ml ascorbic acid) with 10% fetal bovine serum (FBS), changed daily. The cells were then cultured for 1 or 3 days in the absence (control) or presence of LPS (Sigma-Aldrich) at 500 ng/ml ± Dexamethasone at 500 ng/ml. The beads were then dissolved and the cell-associated matrix (CM) was separated from the further-removed matrix (FRM) [7].

PG synthesis: To determine the rate of PG synthesis, the beads were incubated in the presence of 35S-sulfate at 20 μCi/ml during the last 4 hours of culture. The uptake of 35S-sulfate into PGs was determined using a rapid filtration assay [7].

PG degradation: The beads, which were pre-cultured for seven days in complete medium, were then incubated in the presence of 35S-sulfate at 20 μCi/ml for 16 hours. After washing to remove unincorporated 35S-sulfate, the beads were cultured for 1 or 3 days in daily changes of complete medium with or without LPS at 500 ng/ml ± Dexamethasone at 500 ng/ml. At each time point, the content of 35S-PGs remaining in the CM compartment of the matrix was measured and the difference was used to determine the rate of PG degradation (Figs. 3 & 4).

DISCUSSION: The study yielded a number of very significant findings. These may be summarized as follow:

- Exposure of the bovine articular chondrocytes in the beads to dexamethasone in the absence of exogenously added LPS does not have any effect on (i) PG synthesis and (ii) PG loss from the CM and FRM compartments of the matrix.

- Exposure of the beads to dexamethasone led to a slight, although not statistically significant, increase in the total amount of 35S-PGs in the CM accumulating around the cells. Because the MMPs are involved in the normal turnover of radiolabeled PG molecules in the articular cartilage matrix, it is possible that the dexamethasone-induced mild increase in 35S-PG content we observed was, at least in part, the result of a small increase in the half-life of the PGs in the extracellular matrix.

- Dexamethasone used in combination with LPS completely blocked the LPS-induced increased loss of 35S-PGs that accumulated in the CM during the period of culture. This effect manifested itself in spite of the fact that this glucocorticoid did not at any time block the LPS-induced inhibition of 35S-PG synthesis. The finding that dexamethasone can successfully inhibit and/or block the LPS-induced degradation of PGs from the CM compartment of the matrix is exciting.

- The CM compartment is much more sensitive to the effects of LPS ± Dexamethasone on both PG synthesis and degradation than the more voluminous FRM compartment.