Agents and Actions, vol. 17, 3/4 (1985)

0065-4299/85/040320-02$1.90

Ultrastructural and morphometrical studies on the articular cartilage of rats: the destructive effect of Dexamethasone and the chondroprotective effect of RUMALON®

MARLIESE ANNEFELD

Department of Experimental Medicine, Robapharm Ltd., 4006 Basle, Switzerland.

Introduction

In articular cartilage the chondrocytes are responsible for producing and maintaining the extracellular matrix and therefore it is essential that they remain functioning efficiently. Reduced proteoglycan synthesis by the chondrocytes leads to demasking of the collagen fibres and to the onset of osteoarthritic lesions. It is well known that glucocorticoids inhibit proteoglycan and collagen synthesis and such metabolic alterations following glucocorticoid treatment have generally been examined biochemically. Until now, however, no systematic studies have been carried out on the influence of different drugs on the ultrastructure of chondrocytes in the articular cartilage. Using an ultrastructural test system [1] based on analysis of standardised morphometry we are able to systematically examine changes in the organelle systems of chondrocytes under the influence of exogenous agents. The evaluation of large numbers of cells allows changes to be ascertained both quantitatively and qualitatively, and conclusions to be drawn with regard to cell metabolism.

Materials and methods

Dexamethasone as a classical anti-inflammatory agent, and the glycosaminoglycan (GAG)-peptide-complex RUMALON® as a chondroprotecting agent have been studied. The experiments were conducted with male Wistar rats aged 3–4 months at the beginning of the study and weighing approx. 300 g. The rats were injected with 1 mg of Dexamethasone intramuscularly three times at weekly intervals. For the additional chondroprotective therapy the rats were injected with 0.1 ml RUMALON® intramuscularly three times per week. After five weeks the entire cartilaginous tissue was removed from the heads of the knee joints and prepared for electron-microscopy. Electron-microscopic photographs of chondrocytes from the middle layer of cartilage were taken and evaluated in the ultrastructural morphometric analysis system. At least 5 animals per study group were used for the documentation of the chondrocyte ultrastructure. Fifty chondrocytes of the knee joint – half from the femur, half from the tibia – from each animal were examined morphometrically, i.e. at least 250 cells per study group. For standardization and for comparison of the different groups, only medially sectioned chondrocytes were investigated using the nucleus/cytoplasm ratio as the selection criterion. Every measurable cell structure was morphometrically evaluated according to number, length and area. In addition a minimum number of 5000 chondrocytes per group were electronmicroscopically evaluated for viability.

Morphometrical parameters for each study group were expressed as the median value. Groups of values were analysed by the two-tailed Students' T-test and considered significantly different when $p < 0.05$.

Results and Discussion

The normal chondrocyte in the middle layer of articular cartilage is a metabolically active cell and the continuous regeneration of proteoglycans demands a high rate of synthesis of proteins and polysaccharides. This is associated with marked development of the rough endoplasmic reticulum (ER) in lamellar and dilated forms and the presence of prominent Golgi complexes. Dexamethasone brings about massive degenerative changes in the ultrastructure of the vital chondrocyte. The rough ER and the Golgi bodies are markedly reduced following Dexamethasone treatment. Synthesis and secretion of the necessary proteoglycans is no longer assured with this small number of organelles. The balance between anabolic and catabolic processes in the cell shifts in favour of catabolism. The consequences of such changes are demasking of the collagenous fibres and early arthrotic changes in the articular cartilage. The therapeutic administration of RUMALON® partly compensates for the damaging effect of Dexamethasone on the articular cartilage. KALBHEN [2] has reported similar findings. Using light microscopy he demonstrated that RUMALON® caused a significant reduction of the osteoarthritic lesions in rats with experimentally induced osteoarthritis. According to Kalbhen, the chondroprotective effect of RUMALON® is due to the stimulation of anabolic processes of metabolism and to the inhibition of enzymes involved in the degradation of the cartilage. Our results do not point to a stimulation of synthesis by the damaged chondrocyte treated with RUMALON® but to a reduction or even elimination of the degenerative processes seen after corticosteroid administration. The content of lamellar rough ER which is reduced by more than 50% during Dexamethasone treatment is reduced to only 9% after concomitant RUMALON*. The chondrocyte is thus able to synthesize the proteoglycans required for maintaining the

Table
Morphometrical evaluation of chondrocyte ultrastructure after 5 weeks. Influence of Dexamethasone and RUMALON® ('1' = significant differences between control- and the test-groups and '2' = between the Dexamethasone and Dexamethasone + RUMALON® groups, $p < 0.05$).

	Control	Dexamethasone		Dexamethasone + RUMALON®	
Endoplasmic reticulum					
Length (μm)	53.4	1 24.7	1	47.0	2
Dilated area (μm^2)	0.6	0.7		0.73	2
Golgi bodies: Number	3.2	1 2.1	1	2.5	
Mitochondria: Number	11.6	1 13.8	1	14.6	
Total area (μm^2)	1.0	1 0.9	1	1.13	2
Individual area (μm^2)	0.08	1 0.06		0.08	2
Lysosomes: Number	5.2	1 8.1	1	8.9	
Fat vacuoles: Number	0.2	0.3		0.4	
Total area (μm^2)	0.02	0.03		0.04	
Glycogen:					
Cluster area (μm^2)	0.01	1 0.4	1	1.1	2
Mortality rate (%)	4.6	1 15.3	1	10.4	2

integrity of the articular cartilage. KARZEL *et al.* [3] showed a similar effect in fibroblast cultures where the chondroitin sulphate synthesis inhibition induced by corticosteroids could be reversed by RUMALON® treatment. RUMALON® has a normalizing effect on the mitochondrial regression induced by Dexamethasone. Due to the abundance of structurally normal mitochondria after RUMALON® therapy the cell is supplied with sufficient energy for intracellular metabolic processes. It seems that the chondrocyte has a better nutrient supply with RUMALON® treatment and therefore more oxygen is available. Even the regressive changes (glycogen accumulation and increased microfilament content), which occur with Dexamethasone, are only slightly pronounced after concomitant RUMALON® administration. In the normal articular cartilage 4.6% of the chondrocytes in all cartilage zones are dead whilst Dexamethasone treatment markedly enhances the mortality rate of the chondrocytes to a median of 16.3%. Here again RUMALON® acts as a chondroprotecting agent by reducing the cell mortality rate to 10.4%. This study has clearly demonstrated the protective influence of RUMALON® on the articular cartilage damage induced by glucocorticosteroid treatment.

References

[1] M. ANNEFELD, *A new test method for the standardised evaluation of changes in the ultrastructure of chondrocytes*, Tissue Reactions VII (4), 273 (1985).

[2] D.A. KALBHEN, *The inhibitory Effects of Steroidal and Non-Steroidal Antirheumatic Drugs on Articular Cartilage in Osteoarthrosis and its Counteraction by a Biological GAG-Peptide Complex (RUMALON)*, Z. Rheumatol. *41*, 202 (1982).

[3] K. KARZEL, D.A. KALBHEN and R. DOMENJÒZ, *Intractions between corticosteroids and a mucopolysaccharide-containing tissue extract on mucopolysaccharide metabolism of fibroblast cultures*, Pharmacology *2*, 295 (1969).