

Drug effects on arthrosis

Comparison in rabbits of 3 modes of action

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Arthrosis was induced in adult rabbit knees by immobilization in extension. The influence of some intraarticularly injected drugs on the course of arthrosis was studied. Arteparon®, Trasylol®, and Kenacort A10® increased the contents of acidic glycosaminoglycans in the chondrocytes. Only Arteparon had a favorable effect on the integrity of the articular surface.

Arthrosis generally proceeds with remissions and exacerbations. This impedes the evaluation of the effect of a given medicament in a clinical trial. At the cellular level, arthrosis is characterized by increased activity of lysosomal enzymes (acid phosphatase, β -galactosidase, cathepsin), indicative of autodestructive processes and loss of glycosaminoglycans in cartilage matrix and synovia. Moreover, inflammatory signs may be observed.

We have studied the effect on arthrosis induced in the rabbit (Langenskiöld et al. 1979) of three drugs with supposedly different modes of action.

Material and methods

Adult rabbits, type Flemish Giant, were divided into three groups. In the experimental Group and control Group 1, the right knee was immobilized in extension by a polyvinylchloride splint (Langenskiöld et al. 1979), and the animals were killed after 12 weeks. In the experimental Group and control Group 2, drugs were injected intraarticularly in the right knee at weekly intervals from the day of immobilization (Table 1).

Trasylol® (aprotinine) is considered an inhibitor of proteinase activity. Intraarticular application has been claimed to cause a decrease in lysosomal and other enzymes, e.g., lactic dehydrogenase (Czipott and Fenijes 1979). This drug is mainly used in eastern European countries.

Arteparon® is a composite glycosaminoglycan polysulphate. Its structure is related to that of the glycosaminoglycans in cartilage matrix. Intraarticular administration of Arteparon leads to an increase of glycosaminoglycans in cartilage matrix and a higher viscosity of synovial fluid (Brederfeld 1978, Dustman et al. 1974).

Kenacort A10® is a glucocorticoid without mineralocorticoid activity. It is used because of its antiphlogistic action. After intraarticular application, there is an initial decrease in the synthesis of proteoglycans, but after cessation of medication synthesis increases ninefold (Behrens et al. 1976).

The weekly doses were 300 IE Trasylol, 17 mg Arteparon, and 0.3 mg Kenacort A10.

Table 1. Experimental groups and number of rabbits

	Drug	Im- mobili- zation	A Trasylol	B Arteparon	C Kenacort
Experimental group	+	+	6	6	5
Control group	-	+	5	6	5
Control group	+	-	4	4	5

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The knee joints of both sides were dissected and the articular surfaces were photographed. The knees were then fixed in 10 percent formalin in phosphate buffer (pH 7.4) for 3 days and subsequently demineralized in 20 percent formic acid with 5 percent sodium citrate. The rate of demineralization was assessed radiographically (48 KV, 0.7 mA, 1 s, distance 0.7 m). After demineralization the specimens were rinsed, dehydrated in graded alcohol, brought to methyl benzoate, and transferred to 1 percent and 2 percent solutions, respectively, of celloidin in methylbenzoate. Subsequently, they were brought via chloroform and toluene to melted paramat (56°C) under vacuum and embedded. The whole procedure took 1-2 months. Serial sagittal sections (10 μ m) of the joints were prepared in a LKB Multirange microtome. Sections were stained with hematoxylin-eosin, PAS for neutral polysaccharides and Alcian Blue 8GX (pH 1.0) for acid glycosaminoglycans according to Lipiello et al. (1979) and Stockwell and Sprinz (1981).

Three standardized sites from femur (F 1, 2, 3) and tibia (T 1, 2, 3) were identified in the serial sections. The thickness of the meniscus in the sagittal sections served as an orientational guide. The sections were evaluated semiquantitatively according to criteria (Table 2) proposed by Eronen et al. (1978), Langenskiöld et al. (1979), Michelsson et al. (1977), and Weseloh et al. (1977). The number of clefts and clusters was determined per microscopic field using a 16x objective. The total number of positive signs per section was recorded. Significance was calculated according to van Soest (1972). Moreover, the thickness of the mineralized layer in relation to the total thickness of the articular cartilage was determined.

Table 2. Diagnostic criteria for the state of the articular cartilage

1 Surface	0	normal
	+	lesioned
	++	deep lesion (as far as subchondral bone)
2 Clefts	0	none
	+	1-5 per field
	++	more than 5 per field
3 Arrangement of chondrocytes	0	normal, palisades
	+	unorderly, palisades
	++	unorderly, no palisades
4 Clusters of chondrocytes	0	none
	+	1-5 per field
	++	more than 5 per field
5 Alcian Blue staining of chondrocytes	0	none
	+	moderate
	++	intense
6 PAS. staining of chondrocytes	0	none
	+	moderate
	++	intense

Results

The left control knees of the rabbits did not show any abnormality. All the immobilized knees developed arthrosis. Only Arteparon affected the course of arthrosis (Table 3); the reduction in articular surface lesions was about 35 percent (Figures 1 and 2). No effect of the drugs could be demonstrated with respect to the number of clefts, the arrangements of chondrocytes, or the occurrence of clustering of chondrocytes (Tables 4, 5, and 6).

According to Alcian Blue staining, all three drugs tested increased the content of acid glycosaminoglycans in the chondrocytes (Table 7). PAS-positive material in the chondrocytes was increased only after Trasylol treatment (Table 8). The ratio of calcified cartilage thickness over total thickness was about 10 percent higher at the tibial side compared with the femoral side. The drugs did not influence this ratio (Table 9).

Table 3. Effect on articular surface

Group	X ₁	SX ₁	X ₂	SX ₂	Delta 1/2	S Delta 1/2	Delta 1
A	1.03	0.12	1.30	0.07	-0.27	0.14	+0.01
B	0.62	0.06	1.28	0.09	-0.66	0.11	-0.44
C	1.02	0.13	1.22	0.14	-0.28	0.19	+0.19

X₁ Average score of the six sections.

SX₁ Standard error in the average score.

X₂ Average score in the control group.

SX₂ Standard error in the average score.

Delta 1/2 Difference in average X₁ and X₂.

S Delta 1/2 Standard error in the difference.

Delta 1 Resolution based on the actual number of sections (Tables 3-8).

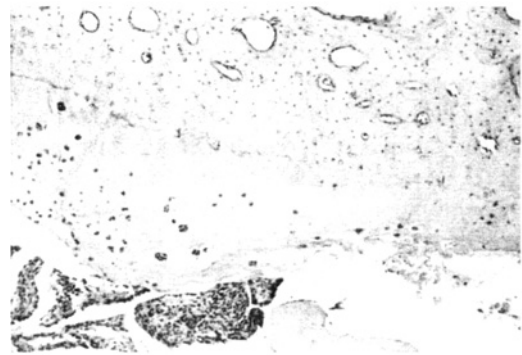


Figure 2

Figure 1. Histologic specimen demonstrating articular cartilage from the tibia after immobilization of the knee joint and administration of Arteparon.

Figure 2. Histologic specimen demonstrating articular cartilage from the tibia after immobilization of the knee joint and no administration of drugs.

Table 4. Number of clefts per field of view

Group	X ₁	SX ₁	X ₂	SX ₂	Delta 1/2	S Delta 1/2	Delta 1
A	0.97	0.10	1.28	0.16	-0.31	0.19	+0.66
B	0.89	0.03	1.11	0.10	-0.22	0.10	-0.03
C	0.93	0.13	0.87	0.18	+0.06	0.22	-0.36

Table 5. Arrangements of chondrocytes

Group	X ₁	SX ₁	X ₂	SX ₂	Delta 1/2	S Delta 1/2	Delta 1
A	1.03	0.15	1.12	0.09	-0.09	0.17	+0.24
B	0.92	0.07	1.00	0.10	-0.08	0.12	+0.15
C	1.10	0.07	1.33	0.09	-0.23	0.11	-0.02

Table 6. Clustering of chondrocytes

Group	X ₁	SX ₁	X ₂	SX ₂	Delta 1/2	S Delta 1/2	Delta 1
A	0.73	0.16	0.45	0.14	0.28	0.21	0.12
B	0.68	0.06	0.94	0.11	-0.26	0.13	-0.12
C	0.67	0.33	0.56	0.13	0.11	0.35	-0.57

Table 7. Alcian Blue staining

Group	X ₁	SX ₁	X ₂	SX ₂	Delta 1/2	S Delta 1/2	Delta 1
A	0.59	0.13	0.71	0.05	-0.12	0.14	-0.39
B	0.44	0.03	0.58	0.14	-0.14	0.14	-0.41
C	0.38	0.07	0.57	0.11	-0.19	0.13	-0.44

Table 8. Increase of PAS.-positive material

Group	X ₁	SX ₁	X ₂	SX ₂	Delta 1/2	S Delta 1/2	Delta 1
A	0.38	0.06	0.57	0.15	-0.19	0.16	-0.50
B	0.30	0.03	0.25	0.06	+0.05	0.06	-0.07
C	0.34	0.08	0.26	0.08	+0.08	0.11	-0.13

Table 9. Ratio of thickness of mineralized articular cartilage over its total thickness. F femur, T tibia

	A	B	C	A ¹	B ¹	C ¹	A ²	B ²	C ²
F/immobile	0.70	0.68	0.69	0.69	0.74	0.73	0.64	0.69	0.69
F/mobile	0.69	0.71	0.72	0.70	0.65	0.72	0.68	0.68	0.70
T/immobile	0.80	0.80	0.75	0.80	0.82	0.81	0.77	0.77	0.78
T/mobile	0.78	0.77	0.75	0.74	0.81	0.78	0.72	0.77	0.75

Discussion

Our experiments confirmed that the procedure introduced by Langenskiöld et al. (1979) consistently caused arthrosis within 3 months. The location of the tidemark between unmineralized and mineralized articular cartilage was not altered in our rabbit model, though the contrary has been stated (Michelsson et al. 1977, Verbruggen and Veys 1977). There was no evidence that subchondral ossification took place at the expense of mineralized cartilage. Thus, the location of the tidemark does not seem to be a reliable criterion for judging the effect of drug therapy. The most clear-cut finding was that all three drugs produced a higher content of glycosaminoglycans in the chondrocytes. Whether an increase of Alcian Blue-positive material is paralleled by an augmented release of glycosaminoglycans into the intercellular matrix remains to be studied. In

principle, it is also possible that higher intracellular contents are the result of inhibited release to the surroundings of the chondrocyte. The same question remains as to the increase in PAS-positive material in chondrocytes after Trasylol administration.

When the results are evaluated in the light of possible clinical relevance, the effect on the integrity of the articular surface deserves particular attention. Only Arteparon appeared to exert a favorable effect. This may be explained by inhibition of lysosomal enzymes and stimulation of the metabolism of cartilage cells and synovial cells (Dustman et al. 1974). The results reported are based on intraarticular application of drugs during the initial phase of arthrosis. This implies that the relevance of the findings may not be found in the area of therapy of far advanced arthrosis, but in early prevention of the condition.

References

- Bjelle A. Content and composition of glycosaminoglycans in human knee joint cartilage. Variation with site and age in adults. *Connect Tissue Res* 1975;3(2):141-7.
- Breederveld R S. Arteparon en gewrichtsklachten. *Geneeskd Sport* 1978;11:141-7.
- Czipott Z, Fenyés T. Lysosomale Enzymveränderungen infolge intraartikulärer Behandlung mit polyvalenten Proteinase-Inhibitoren. *Z Orthop* 1979;117(1):83-7.
- Dustmann H O, Puhl W, Martin K. Der Einfluss intraartikulärer Arteparoninjektionen bei Arthrose. Tierexperimentelle Untersuchungen. *Z Orthop* 1974;112(6):1188-96.
- Eronen I, Videman T, Friman C, Michelsson J E. Glycosaminoglycan metabolism in experimental osteoarthrosis caused by immobilization. *Acta Orthop Scand* 1978;49(4):329-34.
- Langenskiöld A, Michelsson J E, Videman T. Osteoarthritis of the knee in the rabbit produced by immobilization. Attempts to achieve a reproducible model for studies on pathogenesis and therapy. *Acta Orthop Scand* 1979;50(1):1-14.
- Lippiello L, Johnson M E, Mankin H J. Amino sugar-containing macromolecule distribution and metabolism in articular cartilage from osteoarthritic human hips. *Arthr Rheum* 1979;22:635-6.
- Michelsson J E, Videman T, Langenskiöld A. Changes in bone formation during immobilization and development of experimental osteoarthritis. *Acta Orthop Scand* 1977;48(5):443-9.
- van Soest J. *Elementaire Statistiek. Handleiding voor het college toegepaste Statistiek A.* Delftu Uitgeverij B V, Delft 1972:95.
- Stockwell R A, Sprinz R. Glycosaminoglycan content and cell density of rabbit articular cartilage in experimental lipoarthrosis. *J Anat* 1981;133(2):309-15.
- Thyberg J, Lohmander S, Heinegård D. Proteoglycans of hyaline cartilage: Electron microscopic studies on isolated molecules. *Biochem J* 1975;151(1):157-66.
- Verbruggen G, Veys E M. Influence of sulphated glycosaminoglycans upon proteoglycan metabolism of the synovial lining cells. *Acta Rheumatol Belg* 1977;1(1-2):75-92.
- Weseloh G, Lenz L, Weidlich W. Enzymologische Untersuchungen in der Synovialflüssigkeit bei Gonarthrosen. *Arch Orthop Unfallchir* 1977;88(2):217-23.