Acta orthop. scand. 44, 249–255, 1973

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EFFECT OF TRANEXAMIC ACID (CYKLOKAPRON®) ON THE SYNTHESIS OF CHONDROITIN SULPHATE AND THE CONTENT OF HEXOSAMINE IN THE SAME FRACTION ON NORMAL AND DEGENERATED JOINT CARTILAGE IN THE RABBIT

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Accepted 27.xi.72

ε-aminocaproic acid (EACA) is a potent inhibitor of proteolytic enzymes (Alkjaersig et al. 1959, Ali 1964, Weissmann & Spilberg 1968, Aoike et al. 1969). EACA is demonstrable in articular capsules and synovia for up to 16 hours after intravenous injection (Ahlberg 1970).

Trans-4-amino-methyl-cyclohexane-carboxylic acid, tranexamic acid (AMCA), is a much stronger inhibitor of the activation of plasminogen *in vitro* than EACA (Nakahara et al. 1966). These two inhibitors have been used in the treatment of rheumatoid arthritis and osteoarthritis with an apparently good effect (Nakahara et al. 1966, Aoike et al. 1969).

A good correlation has also been claimed (Whitehouse & Boström 1961) between the anti-rheumatic effect of a drug and its ability to inhibit the biosynthesis of mucopolysaccharide sulphates in articular cartilage.

AMCA (Cyklokapron, supplied by AB Kabi, Stockholm, Sweden) has a strong inhibiting effect on the conversion of plasminogen to plasmin.

The purpose of the present investigation was to assess the effect of AMCA on the synthesis of chondroitin sulphate and the content of hexosamine in the same fraction on normal and degenerated articular cartilage.

MATERIAL AND METHODS

Five full-grown rabbits (Group I) weighing 2.1 to 2.4 kg (mean 2.3 kg) were used. One of the knee joints was operated upon according to a method (Hulth et al. 1970) for making the knee joint unstable. The other knee joint served as a control. The animals were killed 3 months later. Four hours before sacrifice 20 μCi ^{35}S as $Na_2{}^{35}SO_4$ (Radiochemical Center, Amersham, aqueous solution, carrier-free) was injected intra-articularly into both knees.

Another group (II), consisting of 10 full-grown rabbits weighing 3.0 to 4.4 kg (mean 3.7 kg), were operated upon with the same method. The unoperated knee served as a control. From the first postoperative day the animals were given 0.5 g Cyklokapron a day by mouth 5 days a week throughout the experimental period. The Cyklokapron tablet was grasped with curved forceps and placed on the posterior part of the tongue to elicit the swallowing reflex. One of the animals offered difficulties and was therefore excluded from the material.

After 3 months the animals were sacrificed by intravenous injection of a lethal dose of Nembutal. 20 μCi ^{35}S was injected intra-articularly into both knees of the animals 4 hours before they were killed. Immediately after the animals had been killed the articular cartilage was dissected from the tibial and femoral condyles of both knee-joints. The cartilage was removed as carefully as possible with a knife, care being taken not to include other tissue. The cartilage was immediately placed in acetone and dehydrated for a week, during which time the acetone was changed 3 times. The preparation was then weighed (dry weight).

Glycosaminoglycans were extracted from the cartilage by digestion with papain in a phosphate buffer containing cysteine and disodium ethylene diamine tetra-acetic acid, as recommended by Scott (1960), but with a minor modification according to Hjertquist (1964). Chondroitin sulphate was precipitated from the digested substance by excess of cetylpyridine chloride (cpc) as a water-insoluble cetylpyridin complex (Scott 1960). The hexosamine content of isolated polysaccharide fractions was determined by the Elson and Morgan reaction as modified by Blix (1948) and Antonopoulos et al. (1964). Samples were hydrolysed in 6 N HCl for 8 h on a boiling water bath, with subsequent removal of the HCl *in vacuo* in a desiccator over sodium hydroxide pellets. Determination of radioactivity was made in a Packard Tri-Carb liquid scintillation spectrometer (Packard Instr. Company Inc.). Each mg of the samples was diluted in 0,5 ml distilled water. To 0,5 ml of this solution was added 3,5 ml distilled water and 5 ml of Instagel.

RESULTS

In Group I the amount of hexosamine, precipitated as chondroitin sulphate-cpc-complex per amount of tissue was significantly decreased in the degenerated cartilage, (Table 1). The synthesis of chondroitin sulphate per amount of hexosamine was significantly increased in the operated joints.

In Group II the amount of hexosamine in articular cartilage on the operated side did not differ from that on the control side. The synthesis

of chondroitin sulphate was increased in the degenerated articular cartilage, but the difference was not significant (Table 2).

Table 1. Concentration of hexosamines (hex.am.) and synthesis of chondroitin sulphate (ch.s.) in untreated group (I).

Cartilage	γ hex.am./mg	cpm ch.s./mg	cpm ch.s./mg hex.am.
Controls	16.35 ± 1.66	122.5 ± 28.9	7.96 ± 1.92
Degenerated	9.70 ± 0.72	150.8 ± 41.4	17.14 ± 2.63
	.05>p>.01	.6>p>.5	.05>p>.01

Table 2. Concentration of hexasamines and synthesis of chondroitin sulphate in group treated with Cyklokapron (II).

Cartilage	γ hex.am./mg	cpm ch.s./mg	cpm ch.s./mg hex.am.
Controls	28.12 ± 1.91	1.74 ± 0.90	0.07 ± 0.03
Degenerated	31.22 ± 1.72	4.48 ± 1.74	0.16 ± 0.07
	.3>p>.2	.2>p>.1	.3>p>.2

Table 3. Concentration of hexosamines and synthesis of chondroitin sulphate in control joints (left) in treated and untreated groups.

Cartilage	γ hex.am./mg	cpm ch.s./mg	cpm ch.s./mg hex.am.
Untreated	16.35 ± 1.66	122.5 ± 28.9	7.96 ± 1.92
Treated	28.12 ± 1.91	1.74 ± 0.90	0.07 ± 0.03
	.01>p>.001	p>.001	p>.001

Table 4. Concentration of hexosamines and synthesis of chondroitin sulphate in operated joints (right) in treated and untreated groups.

Cartilage	γ hex.am./mg	cpm ch.s./mg	cpm ch.s./mg hex.am.
Untreated (I)	9.70 ± 0.72	150.8 ± 41.4	17.14 ± 2.63
Treated (II)	31.22 ± 1.72	4.48 ± 1.74	0.16 ± 0.07
	p>.001	p>.001	p>.001

In the cartilage of the operated (Table 4) as well as of the unoperated knee (Table 3) the amount of hexosamine was significantly larger and the synthesis of chondroitin sulphate was significantly decreased in the group treated with Cyklokapron and regarding to the synthesis

of chondroitin sulphate both in relation to the amount of tissue and to the amount of hexosamine.

DISCUSSION

Experimental degenerative changes can be induced in articular cartilage by various procedures such as compression of the articular surfaces (Salter & Field 1960, Crelin & Southwick 1964, Ginsberg et al. 1969) or incisions of the articular cartilage (Carlson 1957), but such methods often produce a rather rapid degeneration of the articular cartilage. In the method used in the present investigation relatively slow progressive degeneration of the articular cartilage was induced by the instability of the knee joint in full-grown rabbits. The degenerative changes produced resemble those seen in human osteoarthritis according to Collins (1949).

In conditions produced by proteolytic enzymes, such as streptolysin S and in hypervitaminosis A, the lysosomal membranes become labile and the cartilage matrix is broken down. Certain drugs such as steroids, acetylsalicylic acid and other antiphlogistic preparations used in the treatment of joint diseases, act upon the function or diminish the release of substances from the lysosomes (Weissmann 1966).

Weissmann & Spilberg (1968) put forward a theory according to which protease in the lysosomes of the chondrocytes can break down cartilage matrix by acting upon the protein-polysaccharide complex.

In an *in vitro* investigation of bovine nasal cartilage the same authors have showed that EACA has a retarding effect on the breakdown of cartilaginous matrix. It is not known with certainty whether lysosomes play any role in the aetiology or pathogenesis of osteoarthritis.

Earlier studies by other investigators have shown a decrease in the hexosamine in degenerated cartilage (Matthews 1953, Ginsberg et al. 1969, Mankin & Lippiello 1970, Bjelle et al. 1972, Hjertquist & Lemperg 1972). Such a decrease was also found in the present study. Articular cartilage is capable of a certain regeneration, as judged from increased DNA-synthesis, which has been shown in degenerative joint diseases in man and in animals (Mankin & Lippiello 1970, Hulth et al. 1972, Telhag 1972, Telhag & Gudmundson 1972). This increased synthesis is accompanied by an increased synthesis of glycosaminoglycans (Collins & McElligott 1960, Bollet 1969, Mankin & Lippiello 1970). In the present investigation the synthesis of chondroitin sulphate was found to be increased, and this, too, must be regarded as a sign of regeneration of the cartilage.

Cyklokapron was used in a daily dose corresponding to, on the average, 140 mg/kg body weight. This dose slightly exceeds the maximal dose recommended for humans, which is about 120 mg/kg body weight (Nilsson & Rybo 1967).

No significant differences in amount of hexosamine or synthesis of chondroitin sulphate were found between the operated and non-operated knees in the group treated with Cyklokapron. This may, perhaps, be explained by the assumption that Cyklokapron inhibits the activation of plasminogen to plasmin and/or by stabilizing the lysosomal membranes of the chondrocytes and thereby prevents the escape of enzymes and consequent breakdown of the ground substance.

The present investigation showed that the amount of hexosamine was significantly larger in the group treated with Cyklokapron than in the untreated group. In addition, the synthesis of chondroitin sulphate was markedly reduced in the treated group. The reason why the amounts of hexosamine were larger might be the same as that reported above. If so, it would mean elimination of the factor possibly stimulating the synthesis of chondroitin sulphate in degenerative joint disease.

SUMMARY

Full-grown rabbits were used. One knee was operated upon to produce degenerative joint disease. One group of animals was given AMCA by mouth. The findings showed that AMCA markedly reduces the synthesis of chondroitin sulphate and increases the amount of hexosamine in this fraction in the articular cartilage of joints with and without degenerative changes.

This suggests that the preparation prevents the degradation of the cartilage matrix either by preventing the activation of plasminogen to plasmin and/or by stabilizing the lysosomal membranes of the chondrocytes and is therefore apparently of value in the treatment of degenerative joint diseases.

ACKNOWLEDGEMENTS

The author thanks Professor Sven Gardell, Department of Physiological Chemistry II, Chemical Center, University of Lund, Sweden. Financial support was obtained from AB Kabi, Riksföreningen mot reumatism, Ulla och Gustaf af Ugglas fond and the Swedish Medical Research Council (Project No 17X-2436).

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