Inhibitory effect of antiproteoglycan serum on the biosynthesis of proteochondroitin sulfate in calf rib cartilage in vitro[1]

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Summary. Incubation of calf rib cartilage slices with antiproteoglycan serum elevates CO_2-production, but inhibits biosynthesis of proteochondroitinsulfate and of total protein. Absorbing antiserum with proteoglycan abolishes these effects.

In animals osteoarthritis can be induced by repeated applications of antiproteoglycan immune globulin showing histochemically decreased amounts of 'chondromucoprotein' in articular cartilage, as well as an increased radiosulfate incorporation[2]. Similar findings are common with human osteoarthritis[3-5]. Furthermore, the entry and persistence of antigen-antibody complexes in surface layers of articular cartilage appear to be accompanied by a loss of proteoglycans[6,7]. In the paper presented, the effect of antiproteoglycan serum on metabolic functions of cartilage cells is studied in a semi-in-vitro system to get earlier information on the immune globulin effect.

Materials and methods. Antigen. Proteoglycan was precipitated with ethanol and potassium acetate from homogenized calf rib cartilage[8]. It consists of (μmole/g) uronic acid 970, galactosamine 960, glucosamine 80, sulfate esters 1050, galactose 170, pentoses 40, sialic acid 20, and protein 270 mg/g containing collagen 15 mg/g. A collagen fraction was prepared from guanidin-extracted cartilage slices with collagenase[9] separating off proteoglycans on Sepharose 6B. *Antiproteoglycan serum* was obtained by injection of rabbits 4 times with 2 ml Freund's complete adjuvant mixed with 20 mg proteoglycan dissolved in 1 ml saline. It precipitated its antigen with 4 lines in the Ouchterlony double diffusion test[10] exhibiting titers of 1:2 to 1:4; after proteoglycan treatment with testes hyaluronidase 4–5 lines were obtained. After absorption with the collagen fraction 1–2 lines disappeared and after absorption with proteoglycan no line could be detected. The sera of 12 untreated rabbits served as controls.

In vitro experiments. 300 mg freshly prepared slices of calf rib cartilage (50–100 μm thick) were incubated at 37 °C with 0.8 ml antiserum or control serum, respectively, plus 2.2 ml modified Krebs-Ringer-bicarbonate-glucose buffer (medium) under anaerobic conditions (95% N_2, 5% CO_2)[11]. The CO_2-production was measured manometrically[11]. For biosynthesis studies slices were preincubated for 2 h with the sera and incubated for 1.5 h with 2 radioactive precursors (double labelling)[12]. From the slices, total protein and chondroitin sulfate peptides were isolated by papain digestion and precipitation with cetylpyridinium chloride[11]. Analysis of constituents has been described[8,11].

Results and discussion. The incorporation experiments with radioactive precursors indicate an inhibition of the proteoglycan biosynthesis, most probably at the sulfation

1 Acknowledgment. This work was supported by grant Kl 193/9 and 10 from the Deutsche Forschungsgemeinschaft.

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Effect of antiproteoglycan serum on biosynthesis of proteochondroitin sulfate and total protein in calf rib cartilage

Serum added	Chondroitin sulfate peptides: Sulfate esters ^{35}S dpm/μmole SO_4^{2-}		Hexosamine residues ^{3}H dpm/μmole hexosamine		Serine residues ^{14}C dpm/μmole Ser		Leucine residues ^{3}H dpm/μmole Leu		Total protein: ^{14}C-Ser dpm/mg protein		^{3}H-Leu dpm/mg protein	
Control (n = 5–10)	4200 ± 880	100%	1465 ± 280	100%	1105 ± 110	100%	22470 ± 3660	100%	12490 ± 1860	100%	66690 ± 7490	100%
Antiproteoglycan (n = 3–6)	1880[a] ± 410	45%	910[a] ± 170	62%	740[c] ± 110	67%	13870[b] ± 3435	62%	8340[a] ± 500	67%	36330[c] ± 3160	54%
Antiproteoglycan absorbed with collagen (n = 2–3)	2150[a] ± 290	51%	850[b] ± 190	58%	730[b] ± 170	66%	–	–	7555[a] ± 390	60%	39670[b] ± 13140	59%
Antiproteoglycan absorbed with proteoglycan (n = 3–6)	4180 ± 530	99%	1280 ± 160	87%	980 ± 180	89%	19340 ± 3350	86%	9960 ± 420	80%	50970 ± 34680	76%

Mean values ± SD; [a] $p < 0.05$; [b] $p < 0.02$; [c] $p < 0.01$. In vitro double labeling[12] with 20 μCi $^{35}SO_4^{2-}$, carrier free, plus 20 μCi L-leucine-4,5-^{3}H (31.9 Ci/mmole) or 5 μCi L-serine-^{14}C (U, 128 mCi/mmole) plus 10 μCi D-glucosamine-6-^{3}H (7.3 Ci/mmole).

process which exhibits the strongest inhibition (table), since feedback mechanisms exist between sulfation and elongation processes of the polysaccharide chain and, in addition, between chondroitin sulfate side-chain synthesis and the formation of the side chain protein linkage region[11,12]. Biosynthesis of total protein is also inhibited as proteoglycans represent about one half of cartilage dry weight[13]. The effect appears to be specific for antiproteoglycan serum, since it abolishes after absorbing the antibodies with proteoglycan, but not with cartilage collagen. Data presented could indicate possible receptors at the cell surface of cartilage cells acting with these antibodies or with soluble proteoglycan immune complexes, as has been suggested for the proteoglycan-hyaluronate complex[14]. At present it is still unclear which kind of antigen from the proteoglycan complex[10] produces the antibodies causing this effect.

The decreased biosynthesis capacity runs obviously parallel with an increased anaerobic glycolysis: during incubation rates of CO_2 production increase significantly ($p < 0.001$) up to 125 to 150% of controls (2.23 μmoles/g wet weight); the effect abolishes after absorbing the antiserum with proteoglycan, but not with cartilage collagen. Only after longer incubation (5.5 h) the activity of lactate dehydrogenase rises slightly to 120% of controls in the medium indicating cell damage. The content of carbazole-positive material (probably solubilized proteoglycans) as well as the activity of acid phosphatase exhibit minimal changes in the medium.

The data resemble findings observed with human rheumatoid arthritis: in synovial fluids a rise in pCO_2 and in concentration of lactic acid[15,16] as well as elevated cytoplasmic enzyme activities[17] (e.g. lactate dehydrogenase). The production of proteoglycans appears to be diminished with human rheumatoid synovial cells[18] and in joint cartilage of experimental hyperergic arthritis with later states[19]. However, in human rheumatoid arthritis, antiproteoglycan antibodies have not yet been detected, although a potential cell-mediated immune response to proteoglycan antigen has been demonstrated[20]. Finally, it has to be stated that early in vitro effects of antiproteoglycan serum on cartilage cells differ from the changes observed with animal osteoarthritis, which shows increased proteoglycan biosynthesis and loss of proteoglycan from articular cartilage only after repeated injections of antiproteoglycan immune globulin[2].

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The hemolytic activity of heterocyclic N-alkyl amine oxides

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Summary. The N-alkyl derivatives of morpholine-, pyrrolidine-, piperidine- and perhydroasepine-N-oxides caused the rapid, temperature-dependent, hemolysis of human red blood cells. The most hemolytic were the amine oxides with alkyl groups having 14–18 carbon atoms.

The N-alkyl derivatives of saturated heterocyclic amine oxides represent the biodegradable nonionic amphiphiles[1,2], possessing a significant antimicrobial activity[3,4]. Our recent studies on the mode of action of these relatively nontoxic compounds[5] revealed that disorganization of membrane structure after interaction of cells with amine oxides is primarily responsible for their antimicrobial activity[4]. This activity was found to be significantly dependent on the chain length of the hydrophobic alkyl, while it was only slightly influenced by other substituents of polarized N-oxide group[4]. In this paper we describe the effects of homologous series of 4-alkylmorpholine-N-oxides and some other amine oxides with an identical side chain but different basic structures on the stability of plasma membrane of human red blood cells.

Materials and methods. Washed red cells were prepared by diluting fresh human blood with 3 vol. of cold 0.154 M sodium chloride and centrifuging the suspension for 10 min at 1000 × g in a refrigerated centrifuge. The resulting pellet was washed twice and the final pellet of red cells was diluted to about the original volume with 0.154 M sodium chloride.

For measurement of hemolytic activity, the standard incubation mixture at 37 °C contained 0.154 M NaCl, 10 mM Tris-HCl, erythrocytes ($20–40 \times 10^6$ cells per ml) and amine oxide (at indicated concentration), final pH 7.45. At the time indicated an aliquot of mixture was centrifuged at 1000 × g for 2 min and the degree of hemolysis was evaluated by determining of the amount of hemoglobin released in the supernatant liquid spectrophotometrically at 537 nm[6]. The hemoglobin concentration in supernatant of red cells lysed in 10 mM Tris-HCl

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