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BRIEF COMMUNICATION

Proteoglycan clearance in rat femoral cartilage exposed to 6 ATA

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Persliden B, Röckert HOE. Proteoglycan clearance in rat femoral cartilage exposed to 6 ATA. Undersea Biomed Res 1980; 7(3):241–245.—Sprague-Dawley rats were exposed to air at 6 ATA. The decompression time was varied from 30 s to 6 h, resulting in a total pressure time interval from 0–24 h. The amount of rat femoral cartilage proteoglycans was determined by measuring the uronic acid content of the cartilage after papain digestion. The amount of proteoglycans decreased linearly with time of total pressure (decompression time included). No correlation with decompression time was found. It is discussed whether the clearance of proteoglycans is because of a decreased cellular synthesis, assuming the chondrocytes maintain an unchanged degradation activity, or because of an enhanced extracellular transport facilitated by the high pressure.

proteoglycan
rat
cartilage
pressure

One of the most common symptoms in decompression sickness is localized pain in the region of major joints. Because the hyaline cartilage itself is devoid of nerves, the joint pain must be caused by air bubbles or accumulation of metabolites in perichondrium, synovial membranes, tendons, and ligaments. Most of the hyaline cartilages are devoid of vessels. In a few studies, however, channels have been reported that allow fluid transport, although their existence is far from common (1). The microanatomy of cartilage thus causes the metabolism of the chondrocytes to be maintained by diffusion of metabolites over long distances.

A number of investigations have been made on bone tissue after hyperbaric exposures in association with aseptic bone necrosis (2, 3). To the authors' knowledge, however, no investigation has been performed to study biochemical changes in the cartilage after exposure to a hyperbaric milieu.

Since all metabolites diffuse through the cartilage ground substance, and since the extracellular proteoglycans act as steric hindrance or ion exchangers, or both (4), it was of interest to study if a hyperbaric milieu had any effect on the joint cartilage proteoglycans.

The aim of the present investigation was to study if changes in the amount of proteoglycans would occur after exposure to air under hyperbaric conditions and after different decompression periods.

MATERIALS AND METHODS

Experimental design

Male rats of the Sprague-Dawley strain with a body weight of 250–300 g were exposed to air at 6 ATA in a pressure chamber. The rats were divided into five groups with five rats in each group. After the decompression, the rats were decapitated and the hyaline cartilage from the femoral heads was dissected out under a microscope (× 13) and pooled for each experimental group. The control group was not exposed to pressure. Group I was exposed to air at 6 ATA for 24 h and rapidly decompressed in 30 s. Group II was exposed to pressure for 18 h and continuously decompressed in 6 h. Group III was exposed to pressure for 24 h and continuously decompressed in 1 h. Group IV was exposed to the same hyperbaric milieu for 24 h and continuously decompressed in 6 h.

Biochemical analysis

Joint cartilage proteoglycans were estimated by measuring the total amount of uronic acid after proteolytic papain digestion (5, 6).

The cartilage heads were allowed to dry for 24 h at 60°C before digestion. Femoral cartilage head (dry wt. 25–30 mg) was cut into small pieces with a sharp pair of scissors and incubated with 1 ml of 100 mmol phosphate buffer (pH 6.5) containing 50 mmol Na_2-ethylenediaminetetracetate (EDTA) and 5 mmol cysteine-HCl at 65°C, on a shaking water bath. Papain, 40 μl, crystallized two times in sodium acetate suspension (Sigma Chemical Co., St. Louis), was added and incubation was performed for 4 h, at the end of which time another portion of 40 μl papain suspension was added. Total incubation time was 8 h. After incubation, 450 μl 100% ice-cold trichloroacetic acid (TCA) solution was added and the proteins were allowed to precipitate for 10 min, then centrifugated at 5000 *g* for 10 min. The pellet was discarded, and the supernatant was dialyzed against 100 vol of distilled water for 24 h. The glycosaminoglycans were precipitated for 48 h by the addition of 4 vol of ethanol that contained 1% potassium acetate and 1% acetic acid. The precipitate was centrifuged at 5000 *g* for 60 min and dried under reduced pressure in a vacuum desiccator. Total glycosaminoglycan content was assayed by measuring the uronic acid content, using the carbazole method (7). Two analyses were performed for both the experimental and control groups.

RESULTS

As can be seen in Fig. 1 there is a disappearance of uronic acid from the cartilage in proportion to the exposed time. The speed of decompression does not seem to affect the rate

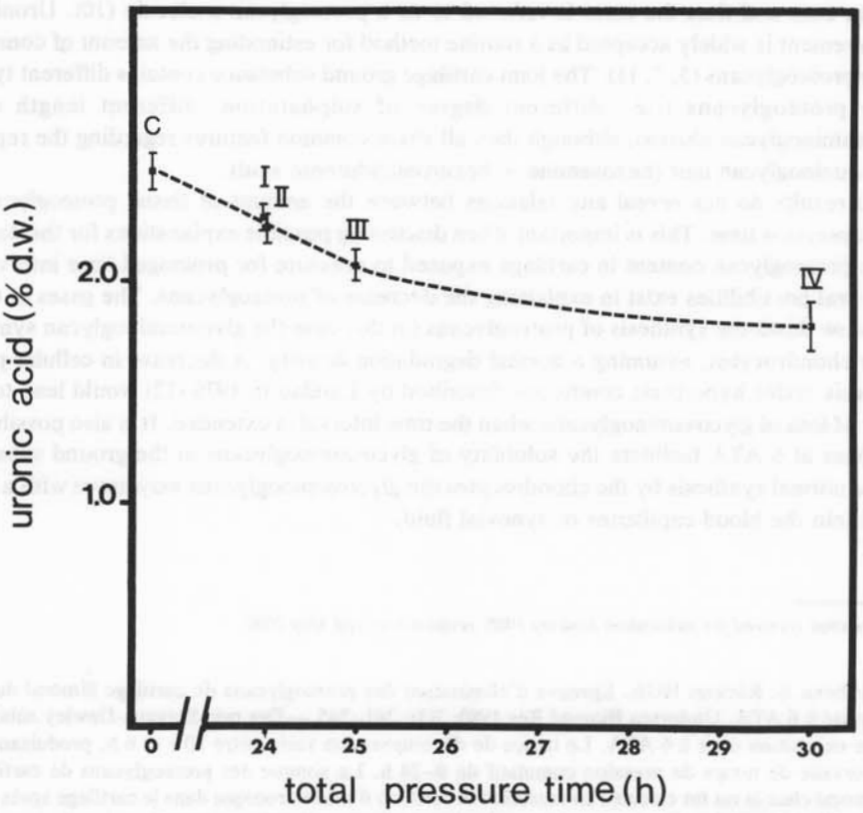

Fig. 1. Decrease of uronic acid in femur head cartilage after different exposure times at 6 ATA. The decrease of uronic acid with time is statistically significant ($P < 0.02$). Amount of uronic acid (% dry wt.): C (control), 2.48; Exp. Group I, 2.28; Group II, 2.20; Group III, 2.06; Group IV, 1.79. For details see MATERIALS AND METHODS section.

of clearance. An allover trend is seen, with a steady decrease in cartilage uronic acid content upon prolongation of the exposure time (including decompression time). The decrease of uronic acid with time is statistically significant ($P < 0.02$) when a correlation coefficient calculation is used.

Uronic acid was also measured in blood samples taken from the animals intracardially but showed no increase in uronic acid content. This could be due to the different compartment size (blood volume/cartilage volume) and does not prove that leakage has not occurred from the cartilage.

To evaluate if the loss of tissue glycosaminoglycans could be more pronounced for any of the different glycosaminoglycan chains that occur in cartilage, electrophoretic separation was performed (8). Preliminary results did not reveal any difference between different types of glycosaminoglycans, but the decrease in joint cartilage seems to include all types of glycosaminoglycans independent of the degree of sulphatation or hexosamine conjugate.

DISCUSSION

The value obtained by determining the tissue content of uronic acid represents the amount of glycosaminoglycans in the tissue (9). These glycosaminoglycans are covalently bonded to a

protein core and thus the mass is referred to as a proteoglycan molecule (10). Uronic acid measurement is widely accepted as a routine method for estimating the amount of connective tissue proteoglycans (5, 7, 11). The joint cartilage ground substance contains different types of these proteoglycans (i.e., different degree of sulphatation, different length of the glycosaminoglycan chains), although they all share common features regarding the repeating glycosaminoglycan unit (hexosamine + hexuronic/iduronic acid).

The results do not reveal any relations between the amount of tissue proteoglycan and decompression time. This is important when discussing possible explanations for the decrease of the proteoglycan content in cartilage exposed to pressure for prolonged time intervals.

Several possibilities exist in explaining the decrease of proteoglycans. The gases at 6 ATA may slow down the synthesis of proteoglycans (in this case the glycosaminoglycan synthesis) in the chondrocytes, assuming a normal degradation activity. A decrease in cellular protein synthesis under hyperbaric conditions described by Landau in 1970 (12) would lead to a net result of loss of glycosaminoglycans when the time interval is extended. It is also possible that the gases at 6 ATA facilitate the solubility of glycosaminoglycans in the ground substance. With a normal synthesis by the chondrocytes the glycosaminoglycans may move with a higher speed into the blood capillaries or synovial fluid.

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Persliden B, Röckert HOE. Epreuve d'élimination des protéoglycans du cartilage fémoral du rat exposé à 6 ATA. Undersea Biomed Res 1980; 7(3): 241–245.—Des rats Sprague-Dawley subirent une exposition d'air à 6 ATA. Le temps de décompression varia entre 30 s et 6 h, produisant un intervalle de temps de pression cumulatif de 0–24 h. La somme des protéoglycans de cartilage fémoral chez le rat fut calculée en mesurant le contenu d'acide uronique dans le cartilage après une digestion de papaïne. Le montant de protéoglycans diminua linéairement en fonction du temps de pression globale (y compris la période de décompression). On ne trouva aucune corrélation avec la durée de décompression. La discussion porte sur le sujet suivant: l'épreuve d'élimination de protéoglycans est-elle dûe à une synthèse cellulaire réduite par les chondrocytes en poursuivant une activité de dégradation continue ou est-elle le résultat d'un transport extracellulaire accru, qui a été facilité par la pression élevée.

protéoglycan cartilage
rat pression

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