Dehydration inhibits matrix synthesis and cell proliferation

An in vitro study of rabbit flexor tendons

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Segments of the deep flexor tendon of the rabbit were exposed to air; the effects of dehydration on in vitro synthesis of proteoglycan, collagen, noncollagenous protein, and cell proliferation were compared with tendon segments that were kept moist with physiologic saline. After 20 min of exposure to air, the tendons lost half and after 40 min all of their ability to synthesize matrix components and to proliferate, whereas irrigated tendons remained viable during the entire experiment.

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Submitted 90-07-20. Accepted 90-10-18.

The flexor tendon is capable of cell proliferation and synthesis of matrix components, such as proteoglycans, collagen, and noncollagenous proteins (Abrahamsson et al. 1989a, b). The metabolic activity and the properties of the tendon vary within the tendon (Abrahamsson et al. 1989a). Further, they are influenced by age and functional demands (Merrilees and Flint 1980, Slack et al. 1984, Viidik 1986, Okuda et al. 1987), and they are possibly regulated by locally or systemically acting agents (Stein 1985, Gauger et al. 1985, Abrahamsson et al. 1991a, b). The metabolic state of the tendon may, however, be disturbed by external conditions, such as trauma and surgery (Birdsell et al. 1966, Manske and Lesker 1984), and drugs (Carlstedt 1986).

We observed that tendon segments for use in vitro experiments dried rapidly and became stiff when left outside the medium and exposed to the circumambient (room) air, as has been described for other collagenous tissues (Morgan 1960). Recently, the deleterious effects of drying on articular-cartilage morphology were reported (Mitchell and Shepard 1989).

We have studied the effects of exposure to air on matrix synthesis and cell proliferation in rabbit flexor tendon, and whether or not these effects could be counteracted by saline irrigation.

Materials and methods

The tendon explant culture and analysis technique have been described (Abrahamsson et al. 1989a). In the present study, 36 intermediate segments of deep flexor tendons from the region of the tendon sheath of the back paws of six rabbits were longitudinally split into 36 pairs. Thus, two matching groups of 36 tendons in each were available for comparison. Each group was further divided into six subgroups, with one tendon per rabbit represented in each (six tendons per subgroup). The tendon segments were placed in separate wells in multidish plates (A/S Nunc, Roskilde, Denmark). During the period of exposure to room air, all 72 tendons were placed in a laminar flow hood at a room temperature of 23 °C and a relative air humidity of approximately 63 percent. One group of 36 tendons were kept moist with physiologic saline solution (two drops/5 min). The exposure to circumambient air was interrupted after 0, 5, 10, 20, 40, or 80 min by adding culture medium to the tendons of the six subgroups, respectively. The medium MCDB 105 (McKeehan et al. 1978) was supplemented with gentamycin (50 µg/mL), ascorbic acid (50 µg/mL), and bovine serum albumin (1 mg/mL); and 1 mL per well was added. The tendons were then incubated for 24 h at 37 °C in a water-saturated atmosphere containing 2 percent CO_2. The medium was replaced with fresh medium supplemented with fetal calf serum (FCS, 10%), and the tendons were incubated for another 24 h. The tendons were labeled for 24 h in fresh medium with FCS and L-^{3}H-proline (10 µCi/mL) and ^{35}S-sulfate (40 µCi/mL; Radiochemical Centre, Amersham, England). The explants were then rinsed and chase-incubated twice for 30 min in fresh medium with supplements and proline (50 µg/mL). The dry weight of each segment was determined after lyophilization.

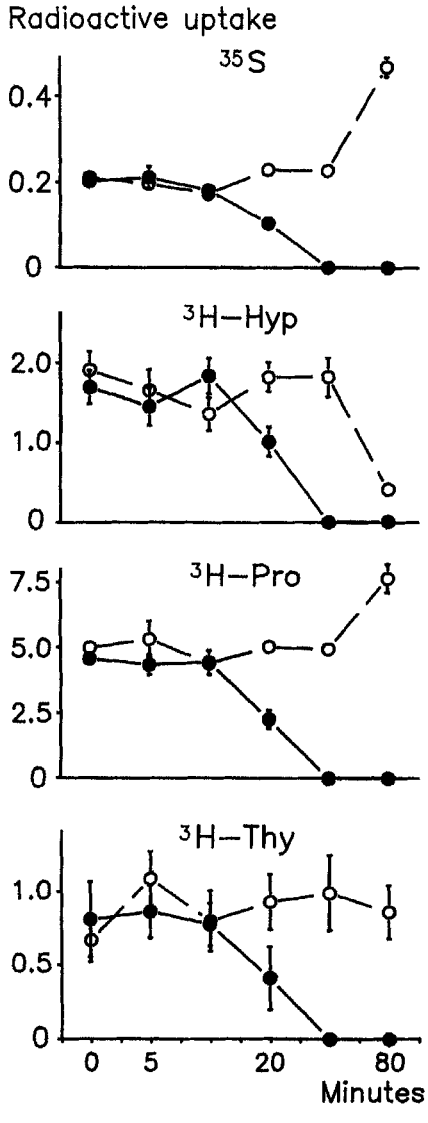

Figure 1. Log-dose effects of time of air exposure (min) on proteoglycan (^{35}S), collagen (^{3}H-Hyp), and protein (^{3}H-Pro) synthesis, and cell proliferation (^{3}H-Thy) in split intermediate segments of rabbit flexor tendon that were not irrigated (●) or were irrigated (○) with a physiologic saline solution. Values are presented as mean radioactive uptake (10^4 dpm/mg) ± SEM (n 6).

Dried tendons labeled with L-^{3}H-proline and ^{35}S-sulfate were hydrolyzed, evaporated to dryness, redissolved, and then separated by HPLC (Lohmander et al. 1976, Abrahamsson et al. 1989a). Radioactivity in peaks corresponding to ^{35}S-sulfate, ^{3}H-hydroxyproline, and ^{3}H-proline were measured. The macromolecular content of ^{35}S-sulfate and ^{3}H-hydroxyproline corresponded to the de novo synthesis of proteoglycan and collagen, respectively. The incorporation of ^{3}H-proline was corrected for the known relative frequency of proline in collagen relative to noncollagenous protein (Diegelman and Peterkofsky 1972). ^{3}H-proline (given in Figure 1 and its text) corresponds to the synthesis of noncollagenous protein. Results of incorporation were expressed as dpm/mg dry weight of tendon tissue.

Cell proliferation was studied in an identically designed experiment comprising another 36 pairs of split intermediate flexor tendons of 6 rabbits (72 tendons). The supplemented culture medium also contained thymidine (5 uM). The tendons were labeled with met-^{3}H-thymidine (10 µCi/mL; Radiochemical Centre) and chase-incubated in fresh supplemented medium, supplemented with thymidine (50 µg/mL). After lyophilization and weighing, the tendons were dissolved in potassium hydroxide: the DNA was precipitated by adding trichloracetic acid, and then was counted in a scintillation counter (Abrahamsson et al. 1989a). The results were expressed as dpm/mg of dry weight tendon tissue.

Statistical analysis

Unless stated otherwise, the results are presented as mean ± standard error of the mean (SEM). Linear regression was calculated by the method of least squares. $LogED_{50}$ refers to the logarithm of the estimated time of exposure eliciting the half maximum effect, and was calculated from the descending part of the regression line. Significance of differences between multiple groups was tested by analysis of variances (ANOVA) and between a control group and multiple groups by Dunett's test. In all the tests, a value of $P < 0.05$ was considered significant.

Results

The rates of matrix synthesis and cell proliferation in dehydrated tendons were reduced by 50 percent after 20 min and by 100 percent after 40 min as compared with their rates at 0–10 min (Figure 1, Table 1). After 40 min of exposure, the rates of synthesis and cell proliferation differed between irrigated and nonirrigated tendons ($P < 0.001$). The cell proliferation in irrigated tendon segments remained unchanged for the experimental period of 80 min. The matrix synthesis in the irrigated tendon

Table 1. The correlation coefficients of the regression lines and the logarithm of the estimated time of exposure eliciting the half maximal effect on the de novo synthesis of proteoglycan (^{35}S), collagen (Hyp), protein (Pro), and cell proliferation (Thy) in rabbit flexor tendon. In all the cases, the correlations were highly significant ($P < 0.001$)

	r	$LogED_{50}$	SD
^{35}S	0.90	1.32	*0.12*
Hyp	0.89	1.31	*0.13*
Pro	0.92	1.30	*0.11*
Thy	0.73	1.31	*0.25*

segments also remained at the same level during the first 40 min; but after 80 min, the synthesis of proteoglycans and noncollagenous proteins had increased ($P < 0.05$) and collagen synthesis had decreased ($P < 0.05$).

Discussion

Even brief exposure to air markedly inhibits in vitro matrix synthesis and cell proliferation in rabbit tendon explants, and the effects are counteracted by moisture with physiologic saline. These time-dependent and deleterious effects appeared soon as compared with the data of Mitchell and Shepard (1989) on rabbit articular cartilage.

The extent of the effects of exposure and the time course may well depend on the thickness of the tendon explant, on the design of the in vitro experiment, and on the air temperature and humidity. These factors will also be of importance when applying our results to the conditions pertaining to tendon surgery in human beings. In the present study, the explants were probably more vulnerable than if the tendons had been left in their anatomic position, as in normal surgical exposure. There was a variation in matrix synthesis in tendons after 80 min of saline irrigation. Immersion of ligaments and annulus fibrosus in water or Ringer's solution cause swelling of collagen fibers and changes in mechanical properties (Viidik and Lewin 1966), and incubation with 0.1 M NaCl for 1 h has been shown to extract larger proteoglycans from ligament tissue (Pearson and Gibson 1982), as well as soluble collagens. Therefore, irrigation with saline may well influence the metabolic state of connective tissues. The extent of these changes or their possible prevention by using other solutions remains to be determined.

Acknowledgements

This study was supported by grants from the Swedish Medical Research Council, the King Gustaf V 80th Birthday Fund, the Medical Faculty of Lund University, the Lundberg Foundation, Malmö General Hospital Foundations, Greta and Johan Kock Foundation, and the Alfred Österlund Foundation.

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