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Elimination of Exogenously Injected Sodium-Hyaluronate from Rabbit Flexor Tendon Sheaths

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Summary: To obtain information about the elimination of Na hyaluronate (NaHe) deposited in flexor tendon sheaths in rabbits, two flexor tendon sheaths from each hind limb were operatively exposed in 15 animals and each filled with one of four different NaHe preparations, namely: 10 mg/ml, MW 2.6 $\times$ 10^6; 10 mg/ml, MW 6.3 $\times$ 10^6; 19 mg/ml, MW 3.0 $\times$ 10^6; and 19 mg/ml, MW 6.5 $\times$ 10^6. One, 3, and 7 days after surgery, the NaHe concentration was measured in frozen specimens from the tendon sheaths. One day after surgery, NaHe concentration in the tendon sheath fluid was close to 10 mg/ml in all groups. Three and 7 days after surgery it was higher in tendon sheaths in which NaHe preparations with a high (19 mg/ml) rather than with a low concentration (10 mg/ml) had been deposited. At 3 days, NaHe concentration was also higher in sheaths that had received a preparation with a high MW (6.5 $\times$ 10^6) than those with a lower MW (2.6 or 3.0 $\times$ 10^6). The findings suggest that within the first 24 h, a dilution of concentrated NaHe preparations takes place, probably as a result of their hyperoncotic properties. After 24 h, there was a slower decline in NaHe concentration when more concentrated solutions (19 mg/ml) and solutions with high MWs (6.5 $\times$ 10^6) were administered. Development of pharmaceutical NaHe preparations with slow elimination from tendon sheaths should reasonably be focused on a solution with a high MW and high concentration. **Key Words:** Hyaluronic acid—Rabbits—Tendons—Tendon sheaths—Pharmacokinetics.

The success of flexor tendon surgery is to a large extent hampered by the formation of adhesions between the healing tendon and the tendon sheath, which results in decreased mobility of the digit. There have been many attempts to diminish adhesion formation, but so far no pharmacologic approach has been unequivocally successful. The only established clinical approach to this problem, is early passive mobilization (9). Recently, there have been a few reports in the literature claiming that instillation of sodium hyaluronate (NaHe) into the tendon sheath immediately before its closure after surgery will reduce the formation of adhesion, by restoring a synovial environment (1,10,11,14–16).

Before further studies are undertaken in an attempt to prevent adhesions after tendon surgery with the use of NaHe, it would seem appropriate to determine how exogenously administered NaHe is eliminated from the tendon sheath. As the formation of peritendinous adhesions seems to begin during the first days after surgery (9), a basic requirement seems to be that exogenously applied NaHe should remain in the peritendinous space at a concentration sufficiently high to have a beneficial effect at least during the critical period when the adhesions form.

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Currently, commercial NaHe preparations have a concentration of about 10 mg/ml and a MW of $\leq 3 \times 10^6$. As the physicochemical properties of NaHe solutions are dependent both on concentration and MW (2,4), it is possible that preparations differing in concentration and molecular weight will have different clinical effects, for example, on account of differences in their pharmacokinetics. In the present study, we have, therefore, evaluated whether different concentrations and molecular weights affected the elimination rate of exogenously administered NaHe from flexor tendon sheaths in rabbit hind paw digits. Four preparations of NaHe were investigated.

MATERIALS AND METHODS

Animals

Swedish Loop rabbits weighing ~2.5 kg were used. They were housed in the laboratory for 14 days before experimentation.

Operative Procedure

Animals were anesthetized with an intramuscular injection of Hypnorm (Janssen, Belgium), 0.5 ml/kg body wt, both hind limbs were shaved, and a 4 cm longitudinal skin incision was made over the flexor tendon of each of the third and fourth digits of both paws (Fig. 1). Tendon sheaths were incised transversely just proximal to the plantar-annular ligament and at the level of the distal interphalangeal joint, and the flexor digitorum fibularis was removed from the tendon sheath. This procedure guaranteed a space in the flexor tendon sheath, which allowed ~0.2 ml of NaHe to be deposited. A 0.4 mm polyethylene catheter (PE 50) was positioned in this space and the tendon sheath ligated at both ends. Approximately 0.2 ml of NaHe was deposited under constant inspection of the tendon sheath in a microscope. In the second part of the study, the exact amount of NaHe administered was determined by the weight of the syringe. The polyethylene catheter was withdrawn and one or two sutures placed over the injection site in the event of visible leakage. The skin was then closed and animals allowed to wake up. In each animal, four digits were operated on and filled with different NaHe preparations.

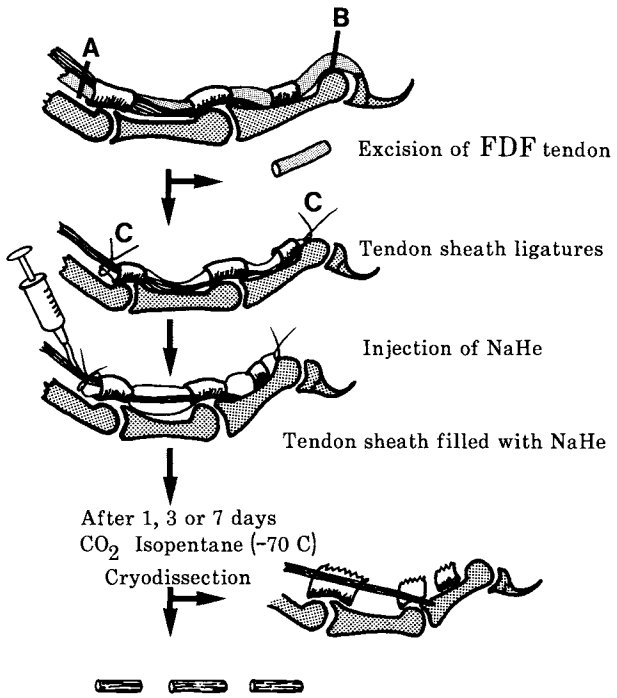

FIG. 1. An artist's view of the technique to determine elimination of exogenously deposited NaHe from the tendon sheath. The tendon sheath was incised at "A" and "B". After removal of the flexor digitorum fibularis tendon in between, the tendon sheath was ligated at "C" and "D", and subsequently injected with NaHe. Full explanation of the technique is given in Materials and Methods.

Animals were killed 1 (two animals), 3 (six animals), or 7 (seven animals) days after surgery. After excision of the skin, the toes were divided at the mid-metatarsal level. As the flexor tendon sheath is tightly attached to the periosteum, it was not possible to isolate the tendon sheath without damage and without leakage of NaHe. The tendon sheath was, therefore, left intact and the whole toe, including the unopened tendon sheath, was quickly frozen in CO_2- chilled isopentane at $-70°C$. The specimen was then dissected in the frozen condition (cryodissected), which enabled the tendon sheath contents to be isolated in a solid state. Several samples were taken and placed in preweighed tubes, which were then reweighed.

In addition, in six separate rabbits, synovial sheath fluid was taken from hind limb tendon sheaths. Synovial fluid was absorbed to a weighed cotton yarn, which was reweighed and put into a test tube with 1 ml phosphate-buffered saline and analyzed as stated below. The reproducibility of this method

was ascertained on samples of NaHe solutions with known concentrations of NaHe.

Determination of NaHe

NaHe concentration in the samples was determined by a slightly modified version of a previously designed specific radioassay (13) based on specific affinity of the cartilage protein, hyaluronic acid binding protein (HABP), for NaHe (12). This method, which is strictly a radioaffinity assay, has previously been modified for protease-treated tissue samples (8) and direct determinations of hyaluronate in serum (5). Weighed samples were extracted with 1 ml of phosphate-buffered saline with the addition of 6% bovine serum albumin. 100 μl of this extract was diluted with the same buffer and analyzed for its content of NaHe according to the principles of the Pharmacia HA50-test (Pharmacia AB, Uppsala, Sweden) (3). Briefly, 100 μl of diluted sample or standard was mixed with 200 μl of ^{125}I-labeled HABP and incubated for 60 min at 4–7°C. 100 μl of 1 mg/ml Sepharose, to which hyaluronic acid was covalently linked (HA-Sepharose), was thereafter added and samples incubated for another 45 min. 2 ml of buffer was added for washing, whereafter the HA-Sepharose was recovered after centrifugation at 2,000 g for 10 min. Bound radioactivity was measured in a gamma counter. Known amounts of NaHe was used to construct a standard curve. Radioactivity was plotted as a function of radioactivity of the samples.

Materials Used

Four different NaHe preparations were administered: A: 10 mg/ml, MW 2.6×10^6; B: 10 mg/ml, MW 6.3×10^6; C: 19 mg/ml, MW 3.0×10^6; and D: 19 mg/ml, MW 6.5×10^6.

The oncotic pressure of these NaHe preparations is mainly dependent on the concentration. Preparations A and B, with a NaHe concentration of 10 mg/ml, are isooncotic, while preparations C and D, with a NaHe concentration of 19 mg/ml are hyperoncotic (2).

Techniques of Tissue Sampling and Hypotheses and Rationale for Calculation of NaHe Concentration in Synovial Fluid

The basic assumption was that the NaHe injected was evenly distributed within the flexor tendon sheath fluid. The aim of the tissue sampling procedure was thus to identify samples that consisted of tendon sheath fluid only. Depending on the visual appearance of the operative site during the dissection, a varying number of samples were taken. In cases where the tendon sheath content was obtained as a large solid specimen, which without any doubt was fully representative, only one sample was taken. More frequently, however, the tendon sheath content was fragmented and/or it was difficult to be sure about the representativeness of the sample. Under those circumstances, the fragmented material was divided in up to seven samples, which were analyzed separately. Eight out of 201 samples from the tendon sheath fluid were eliminated from analysis as the hyaluronic acid content deviated considerably from that of the remaining samples from the same digit.

Statistical Evaluation

Wilcoxon's signed ranks test for paired samples was used on logarithmically transformed values. A difference between groups at the 5% level was considered significant.

RESULTS

The normal concentration of endogenous hyaluronic acid in rabbit hind limb flexor tendon sheath fluid was found to be 3.5 ± 0.7 mg/ml ($n = 6$).

In tendon sheaths filled with NaHe preparations with a concentration of 10 mg/ml, the concentration of NaHe was unchanged after 24 h (Fig. 2). In those filled with NaHe preparations with a concentration of 19 mg/ml, NaHe concentration was about 10 mg/ml after 24 h, i.e., the original concentration had been reduced by 50%. Three days after deposition, the concentration of NaHe was higher in tendon sheaths filled with the two 19 mg/ml preparations than in those filled with preparations with the lower NaHe concentration (10 mg/ml; Fig. 3A). Furthermore, there was a higher concentration of NaHe in tendon sheaths filled with a solution with a MW of 6.5×10^6 than in those filled with a solution with a MW of 3×10^6 (Fig. 3C). Seven days after administration, there was a higher NaHe concentration in tendon sheaths filled initially with more highly concentrated preparations (19 mg/ml) than in those filled with a preparation with a concentration of 10 mg/ml (Fig. 3B). Also, there seemed to be a slightly higher NaHe concentration in those sheaths filled

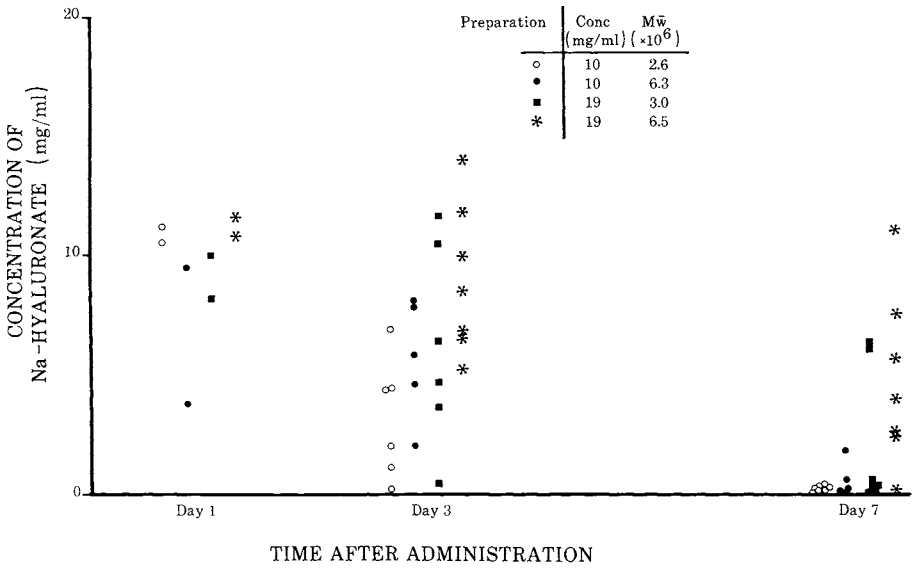

FIG. 2. Concentration of NaHe 1, 3, and 7 days after deposition in rabbit hind paw digit tendon sheaths. Each symbol represents one observation.

with high MW NaHe than in those filled with a preparation of lower MW (Fig. 3D). This trend did not reach statistical significance; however, ($p \sim 0.1$).

DISCUSSION

In the present paper, we report how the concentration of NaHe alters over time after deposition of NaHe preparations with different characteristics into flexor tendon sheaths of the hind limbs of rabbits. In each animal, three to four different NaHe preparations were deposited in different toes. This enabled a paired comparison between different preparations, although a limited number of animals were used. This approach does not, however, provide full information on the pharmacokinetic behavior of NaHe, since the amount of NaHe administered inevitably differed slightly between animals due to interindividual variations of flexor tendon sheath volume and since dilution of the content with wound exudate would most likely influence the result. However, these drawbacks would also prevail in the human clinical setting after administration of NaHe for therapeutic reasons.

The normal NaHe concentration in synovial fluid of the rabbit flexor tendon sheath is ~3.5 mg/ml, which is similar to that in humans (6) and to the concentration in joint synovial fluid of rabbits (Tengblad A, unpublished observations) and humans (7). In the present study, the NaHe concentration had decreased to a value below this normal concentration in many samples by day 7. A possible explanation for this is that at this time the tendon sheath is filled with a wound exudate with a very low endogenous NaHe content, and the NaHe concentration in specimens filled with exogenous NaHe will decline asymptotically to the very low value in the wound exudate.

The present study gives a few ideas as to the mechanism by which NaHe is eliminated from tendon sheath synovial fluid.

Twenty-four hours after administration of a NaHe preparation with a concentration of 10 mg/ml, the concentration of NaHe in the tendon sheath fluid was still close to 10 mg/ml, whereas the NaHe concentration in the tendon sheaths filled with a NaHe preparation with a concentration of 19 mg/ml had decreased from 19 to ~10 mg/ml. As NaHe preparations with a concentration of 19 mg/ml are hyperoncotic, the findings indicate that there is a rapid dilution of these preparations as a result of osmotic forces.

Three days after administration, the NaHe concentration was higher in sheaths filled with preparations with the highest MW. This suggests that the elimination rate is slower for a preparation with a very high MW ($\sim 6.5 \times 10^6$) than for a preparation with a lower MW ($\sim 3 \times 10^6$).

Three and seven days after administration, the NaHe concentration was lower in tendon sheaths filled with a preparation with a concentration of 10 mg/ml than in those filled with a 19 mg/ml preparation. At seven days, the NaHe concentration was virtually zero in many sheaths, especially in the 10 mg/ml groups. As the injected volume is limited, for

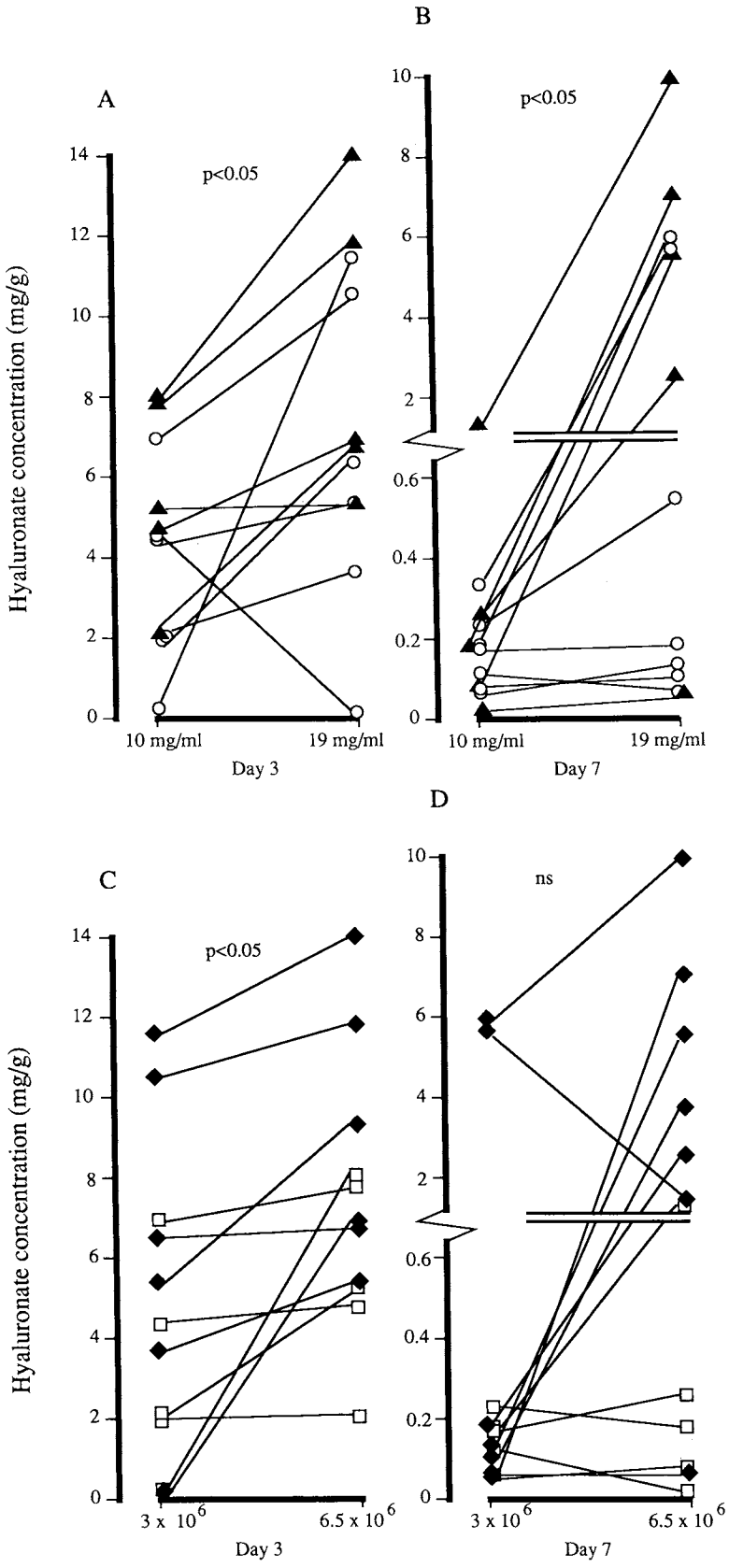

FIG. 3. Concentration of NaHe in synovial sheath fluid 3 and 7 days after deposition in rabbit hind paw digit tendon sheaths. Each pair of symbols originates from one animal. **A** and **B:** Pairs made with regard to differences in the NaHe concentration in the preparations. Closed symbols represent preparations with MW of 6.3 or 6.5 $\times$ 10^6, and open symbols preparations with MW of 2.6 or 3.0 $\times$ 10^6. **C** and **D:** Pairs made with regard to differences in MW in the preparations. Open symbols represent preparations with a NaHe concentration of 10 mg/ml and closed symbols preparations with a NaHe concentration of 19 mg/ml.

practical reasons, the findings suggest that one way to achieve a higher concentration of NaHe in synovial fluid for an extended period of time after surgery is to administer a highly concentrated NaHe solution.

We have established that tissue sampling of frozen specimens can be used to determine the concentration of NaHe in tissue compartments that are difficult to isolate under normal conditions. The study has indicated, further, that administration of NaHe with a very high MW at a high concentration into tendon sheaths results in a higher concentration of NaHe for a longer period of time in synovial sheath fluid than does administration of the type of NaHe preparation that is commercially available today.

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