A Study of the Mechanisms Influencing Ligament Growth

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ABSTRACT: This study investigated the effects of tension and/or exogenous growth hormone on the growth (elongation) of ligaments. In the first experiment, tension was applied to the lateral collateral ligaments of immature rabbits. In the second experiment, a group of skeletally mature rabbits was given exogenous growth hormone while tension was applied to their lateral collateral ligaments. The results revealed that immature rabbit ligaments elongated 140±18% when tension was applied, while "control" ligaments elongated 79±5% (P<.01). In mature rabbits receiving exogenous growth hormone, no significant change was found in the ligament's length with or without the application of tension when compared with controls.

Tension applied to ligaments in immature rabbits can increase ligament growth, indicating that physical forces (tension) may be important in the regulation of ligament growth. The same tension applied to mature rabbit ligaments in combination with exogenous growth hormone did not cause a resumption of growth, indicating that tension and the presence of growth hormone are not the only factors necessary for ligament growth.

Introduction

Abnormalities of ligament growth may be responsible for some childhood conditions of joint contracture or laxity, eg, arthrogryposis and idiopathic scoliosis. Excessive or insufficient ligament growth relative to the underlying bone and cartilage could produce lax or contracted joints. This study investigated two factors to determine their influence on ligament growth.

A review of the literature reveals that much is known about the factors controlling the growth of bone and cartilage, but little is known about the growth of ligaments. Dahners and Muller1 and Frank et al2 revealed that longitudinal medial collateral ligament growth occurs throughout the length of the ligament with the greatest magnitude of growth at the insertion. Wessels and Dahners demonstrated that the rabbit deltoid ligament elongates relatively evenly throughout its length.3

Growth is generally assumed to result from the presence of growth hormone working through the somatomedins. Kibrick et al noted the lack of epiphyseal growth in hypophysectomized rats.4 Daughaday et al5 and Salmon and Daughaday6 found that somatomedin had a direct influence on cartilaginous growth.

The broad scope of growth hormone’s effects throughout the body (through the somatomedins) may encompass ligament growth directly. It is difficult to conceive, however, that direct stimulation of ligament growth (elongation) by a central controlling mechanism such as growth hormone could accurately integrate the ligament’s growth with underlying bone and cartilage. This study hypothesized that there are local factors (such as tension) that directly control ligament elongation during growth.

The first part of the study evaluated the effect of tension on ligament growth with the hypothesis that ligament elongation is coordinated to bone growth by the stretching effect produced by the enlargement of the

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1569
underlying bone. The second part of the study evaluated the effects of growth hormone in conjunction with the effects of increased tension to test the hypothesis that growth hormone would produce a resumption of elongation in a mature ligament under the influence of increased tension.

**Materials and Methods**

New Zealand, white, male rabbits were obtained from a commercial breeder for the study. The first part of the study consisted of three experiments examining the effects of tension on immature rabbits. The second part consisted of two experiments evaluating the effects of growth hormone on ligament growth and contracture in mature rabbits. Both parts of this study had essentially the same surgical procedure, with three variations. All the rabbits were anesthetized with ketamine and acepromazine, and the lateral sides of both knees were exposed using aseptic technique. The lateral collateral ligament (LCL) was isolated and marked with three nylon sutures with an intermarker distance of approximately 2 mm. This intermarker distance was then measured under an operating microscope with a caliper accurate to 0.02 mm and recorded.

Although no specific analysis of measurement accuracy was carried out, 10 repeat measurements of a single interspace all fell within ±0.05 mm and the caliper dial was not visible to the observer at the time he placed the caliper points on the sutures under the microscope. In the "control" ligaments of our study the knees were closed at this point.

The knees designated as "sham" had resection of the central portion of the fibula, and freeing of the fibular head from the tibial metaphysis. A 2-0 silk suture was then passed about the tibia (at its juncture with the stump of the fibula) and sutured to a dental rubber band. Another suture was passed through the fibular head and also tied to the dental rubber band. The rubber band was not placed under any tension. In the knees designated "experimental," the same operation was carried out, with the exception that the dental rubber band was placed under tension by stretching it 1.6 to 1.8 times its original length (approximately force of 1 N, see Fig 1). Postoperatively, all the animals were returned to their cages, where they were provided food, water, and allowed to be active.

At the end of the experiment, all the animals were killed, the knees re-dissected, the intermarker distance remeasured, and the percent growth of the LCL, as revealed by the enlargement of the intermarker distance, calculated.

The first part of the study on the effects of tension on growing ligaments consisted of three different experiments. In experiment 1A, six 6-week old rabbits underwent a sham operation on one leg and a control operation on the other. In experiment 1B, ten 6-week old rabbits underwent an experimental operation on one leg and a control operation on the other. In experiment 1C (performed because of suggestive results in experiment 1B) eight 4-week old rabbits underwent an experimental operation on one leg and a control operation on the other. The rabbits in this part of the study were killed 6 weeks after surgery.

The second part of the study consisted of two more experiments, each lasting 23 days. All the rabbits in these two experiments received oxytetracycline 1M (25 mg/kg) on the day of surgery and on the 21st postoperative day. Each rabbit also received daily subcutaneous injections of ovine growth hormone (1 mg/kg) or an equivalent volume of normal saline.

In experiment 2A, 18 mature (3.5 kg) rabbits underwent a sham operation on one leg and an experimental operation on the other. Nine of these rabbits received daily subcutaneous injections of the ovine growth hormone, while the other nine received subcutaneous injections of normal saline. In experiment 2B, three mature rabbits underwent a control operation on both legs, with all three of these rabbits receiving subcutaneous injections of ovine growth hormone. Each rabbit received its last subcutaneous injection of ovine growth hormone or normal saline on the 21st day. Each rabbit was killed on the 23rd day to allow a maximum effect from the exogenous growth hormone. The knees were then redissected and the lateral collateral ligament intermarker distance remeasured.

After sacrifice the ramus of the mandible was submitted for preparation of undecalcified bone sections. The oxytetracycline labeled bone was examined with a fluorescence microscope. The subperiosteal bone was examined for deposition of the fluorescent label, and the group that received growth hormone was compared to that which received normal saline.

**Results**

Differences between the groups were evaluated for statistical significance using the student's t test. Results are reported as the mean ±1 standard error (Table). In experiment 1A, the sham ligaments elongated 16±4%, while the control ligaments elongated 12±8% (not a statistically significant difference). In experiment 1B, the experimental ligaments elongated 29±11% under the influence of tension from the dental rubber band, while the control ligaments elongated 14±6% (suggestive but not statistically significant). In experiment 1C, the experimental ligaments elongated 140±18%, while the control ligaments elongated 79±5% (statistically significant P<.01).

In experiment 2A, the experimental ligaments on
exogenous growth hormone elongated 3±7%, while the experimental ligaments not having received exogenous growth hormone elongated 5±9% (not a statistically significant difference). Also in experiment 2A, the sham ligaments on exogenous growth hormone shortened 2±5%, while the sham ligaments not having received exogenous growth hormone elongated 4±6% (again not a statistically significant difference). In experiment 2B, the control ligaments grew 1±4% (no significant growth).

In the histologic bone studies on the 21 rabbits in the second part of the study satisfactory sections were obtained in eight of the animals receiving growth hormone and in seven of the control animals. These revealed two separate oxytetracycline lines (indicating new perosteal bone deposition) in all eight of the animals receiving growth hormone and separate lines in none of the animals not receiving growth hormone. The animals receiving growth hormone also had increased hair growth, confirming that the hormone did have an effect.

**Discussion**

This study confirmed the hypothesis that the application of tension to growing ligaments will increase the rate of elongation. The use of 4-week old rabbits (experiment 1C), which are in a more rapid portion of their growth curve, demonstrated this effect much better than the 6-week old rabbits (experiment 1B). Wound healing and scar tissue formation between the tibia and fibular head should have been similar in all groups; the fact that differences were detected indicates that tension did indeed have a significant effect. Perhaps, however, the amount of tension was relatively less in the larger, adult animals or scarring may have more effectively diminished the transmission of tension in the adults, thus making the effect of the surgery in the adults imperceptible. The increased elongation of the ligament in the young animals supports the hypothesis that ligament growth is to some extent controlled by local mechanisms.

The sham operated ligaments surprisingly continued to elongate in experiment 1A, showing that, even without tension, ligaments will elongate in a growing animal. Perhaps this was because of reattachment of the fibular head to tibial metaphysis and a resumption of stretching produced by growth of the tibial physis. This persistent growth in ligaments without tension supports the hypothesis that growth is enabled by a distant mechanism (such as growth hormone), but can be modified by local factors (such as tension). The fact that exogenous growth hormone did not cause resumption of ligament elongation in mature rabbits indicates that growth hormone alone or in combination with tension is not sufficient to cause a resumption of ligament elongation. Perhaps a longer follow-up study
on growth hormone at higher doses may show induced resumption of ligament elongation. The current study was, however, sufficiently long enough to effect both hair and bone growth to a noticeable degree.

References

Editorial Comment
Although the clinical relevance of this article may seem remote, we must recognize the importance of any new information which sheds light on basic physiologic processes. Added to what we do and do not know about growth in general and ligament growth and modeling in particular, this well-controlled study does provide data worth our knowing. There may even be immediate clinical relevance to situations of traumatic tissue and ligament loss in children.