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Massage-induced morphological changes of dense connective tissue in rat's tendon

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Abstract: The aim of the experiment was to determine if possible changes in connective tissue induced by massage could have a positive effect justifing the use of massage in all post-traumatic connective tissue conditions, e.g. tendon injuries. The investigations were performed in a group of 18 Buffalo rats. The rats were divided into two groups (experimental and control). To standardize the massage procedure, it was performed with an algometer probe of 0.5 cm^2 with constant pressure force of 1 kG (9,81 N). To analyse the number and diameter of collagen fibrils, two electron micrographs were performed for each rat of the collected segments of tendons of rat tail lateral extensor muscle. After image digitalization and calibration, the measurements were carried out using iTEM 5.0 software. The number of fibrils, their diameter and area were measured in a cross-sectional area. An increase of the number of collagen fibrils was observed in the tendons of massaged animals compared to the control group. Our study demonstrated that massage may cause a beneficial effect on metabolic activity of tendon's fibroblasts and, in consequence, may be applied for more effective use of massage for the prevention of tendon injury as well as after the injury has occurred. *(Folia Histochemica et Cytobiologica 2013, Vol. 51, No. 1, 103–106)*

Key words: massage, morphological changes, tendon, collagen fibrils, rat

Introduction

Massage is commonly used in sports to prepare the competitor for efforts associated with strenuous participation. It is also applied for prevention and therapy of various injuries occurring in competitors. Its basic task is to improve muscle blood supply and to stimulate regeneration and repair processes in tendons, ligaments and joint capsules [1].

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There are numerous publications confirming beneficial effect of massage on muscles and cardiovascular system [2–4]. However, only few studies concerned the effect of massage, particularly in the context of structural changes occurring after its application [5–8]. Dense connective tissue consists mainly of collagen fibrils characterised by high resistance to stretching. Accordingly, it is possible to transfer pulling forces from muscles to the skeletal system by tendons or to counteract excessive movements in joints by ligaments. This type of connective tissue may undergo constant rebuilding as the result of synthesis of new collagen molecules by fibroblasts.

Some authors demonstrated that the number and diameter of collagen fibrils in rat change with age. An

intensive running and training therapy manual shows relatively well documented factors affecting collagen fibrils [9]. It is known that strenuous sports training can lead to tendon and ligament injury. Moreover, it is well known that massage is commonly used in the process of recovering and rehabilitation in injured competitors. However, there are no studies on the effect of massage on collagen fibril structure, confirming explicitly the usefulness of its application in the therapy and prevention of tendon and ligament injuries. To date, no positive effect of massage has been demonstrated as the factor contributing to the repair of soft tissue injury, particularly of dense connective tissue.

The aim of present studies was to determine if long-lasting massage, using rubbing technique, could change the structure of the tendon's dense connective tissue in ultrastructural examinations by evaluation of the number and diameter of collagen fibrils.

Material and methods

Animals. Investigations were performed in a group of 18 Buffalo male rats aged 15 months and weighing 320–380 g (348 ± 19.5 g, mean and SD). The rats were divided randomly into two groups of 9 each: group 1 — the experimental massaged group (346 ± 20.4 g) and group 2 — the non-massaged group (control, 349 ± 19.7 g).

The study was approved by the Animal Care Ethical Committee of the Clinical Research of the Wroclaw Medical University (Approval Number: 54/2006) and was performed according to the guidelines of the Polish Animal Care and Use Committee.

Massage. Massage was performed using spiral rubbing technique on dorsal-lateral surface of the tail (on the right and left side). The rat tail tendons were selected for the experiment, as they are described in details in literature, taking into account distribution of diameters of the collagen fibrils in relation to the age of the animal [9]. Moreover, the tendons did not undergo strain associated with the activity of the animals. This might have affected the results of the experiment, as it happens in case of e.g. tendons in the extremities. Each rat, both from the experimental (massaged) and control (non-massaged) group, was placed in a narrow cage which did not allow the animal to change its position. There was a hole for the rat's tail at the back of the cage. Thus, it was easily accessible for massage procedure. The massage consisted in deep deformation of tendon fibrils of the tail extensor muscle with the use of spiral rubbing technique on the surface of 1 cm^2 in the middle part of the tail. In order to standardize the massage procedure it was performed with an algometer probe of 0.5 cm^2 surface with constant pressure force of 9.81 N (1kG). The massage was performed 6 min/daily for 60 days. Similar period of connective tissue mobilization was also us by other authors [10]. Duration of a single procedure and the force of applied pressure had to provide deformation of the massaged tissue and at the same time did not exceed the tolerance level to the pressure applied. According to the experience of the authors, the above presented parameters fulfilled the mentioned conditions.

Ultrastructural examination. The EM study was performed on tendons of tail lateral extensor muscle which are a model tissue in this type of studies. After 60 days the experimental and the control group animals were anaesthetized with ketamine Bioketan (10 mg/kg b.w.) and then sacrificed with Morbital (an injectable solution containing 26.7 mg pentobarbitone and 133.3 mg pentobarbitone sodium per 1 mL). The tendons from the middle part of the tail were collected for ultrastructural examination.

The collected segments of tendons of rat tail lateral extensor muscle were fixed in 4% glutaraldehyde in a phosphate buffer [11]], pH 7.4 for 30 min. After cutting into smaller fragments they were placed in fresh fixing agent for 2 hours at 4^0C. Then, the fragments were incubated for 1 hour in 1% osmium tetraoxide in the same buffer, rinsed and dehydrated in series of increased graded alcohol and acetone and then immersed in EPON-812. After polymerization the fragments were cut into semithin fragments and stained with methyl blue to choose appropriate area for electron microscopy. Then, ultrathin sections were cut in transverse cross-sections which were stained with 2% uranyl dioctan and lead citrate. The material was assessed with Tesla BS-540 electron microscope. To analyse the number and diameter of collagen fibrils, two electron micrographs were taken for each rat. After image digitalization and calibration, the measurements were carried out using iTEM 5.0 image analysis software (Soft Imaging System, Münster, Germany). The number of collagen fibrils, their diameter and area were measured in cross-sectional area. The analysis of the distribution of collagen fibril diameters was performed with the division into individual diameter classes: 0–100, 100–200, 200–300, 300–400, 400 > nm.

Statistical analysis. The results of the ultrastructural examinations expressed in the form of numerical values were the subject of statistical analysis with the use of Mann-Whitney U test.

Results

The observation of tendon transverse cross-sections in electron microscopy and the statistical analysis showed differences between the control and the experimental group in their ultrastructure (Figures 1A, 1B). An increase of the number of collagen fibrils was observed in the tendons of massaged animals compared to the

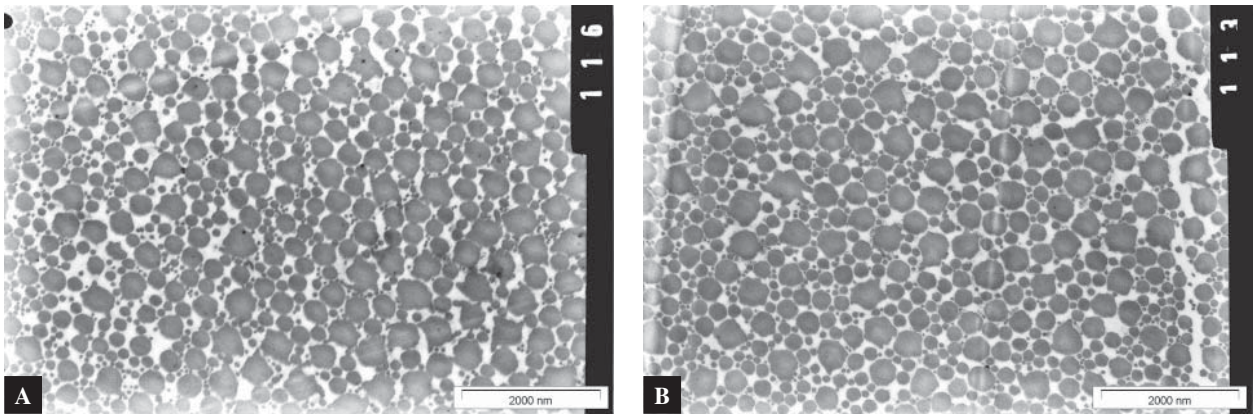

Figure 1A. Transverse cross-sections of collagen fibrils from rat tail tendon of different diameter are visible – the experimental (massaged) group. **B.** Transverse cross-sections of collagen fibrils from rat tail tendon are visible – the control group

Table 1. Comparison of the number and mean area of collagen fibrils in experimental and control group

	Experimental group N = 9[A]	Control group N = 9[B]	Mann-Whitney U Test
Number of collagen fibrils per 16 μm^2 of cross-sectional area			
Mean ± SD	151 ± 118	140 ± 109	P < 0.00001
Median ± Q	114 ± 83	100 ± 74	
Mean area of collagen fibrils [nm^2] 16 μm^2 of cross-sectional area			
Mean ± SD	29261 ± 4015	24699 ± 3262	P < 0.00001
Median ± Q	10217 ± 17697	7807 ± 14628	

[A]The number of all counted fibrils in 9 rats was 5695, [B] the number of all counted fibrils in 9 rats was 4993

control group (Table 1). The mean diameter of collagen fibrils was statistically significantly smaller in massaged tendons than in non-massaged ones (Table 1). Similarly, the mean surface of collagen fibrils was statistically significantly smaller in massaged tendons than in those in the control group (Table 1).

It was found that in the class of fibrils of the largest diameter (> 400 nm), the number of collagen fibrils was statistically significantly lower ($p < 0.001$) in the massaged group than in the control group. In the class of the smallest diameter (< 100 nm) the number of collagen fibrils was statistically significantly higher ($p < 0.001$) in the experimental group than in the control group. In remaining classes 0–100, 100–200, 200–300, 300–400 nm no statistically significant differences were found in the number of fibrils between the massaged group (experimental) and the non-massaged group (control) (Figure 2).

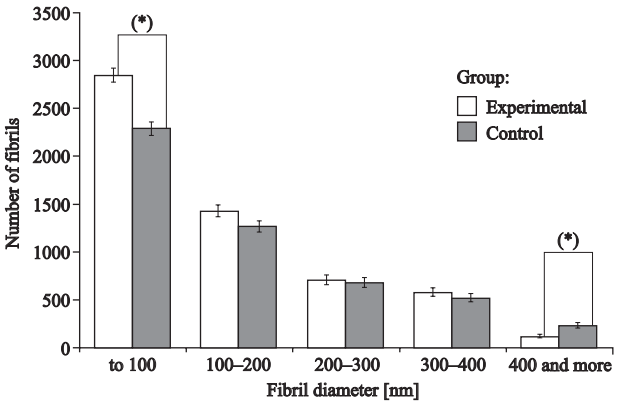

Figure 2. Distribution of collagen fibrils dependent on their transverse cross-section diameter in the experimental group (massaged) and in the control group (*$p < 0.05$)

Discussion

It is known that connective tissue can undergo constant reconstruction associated with numerous factors, such as age or mechanical load. It was demonstrated that the diameter of collagen fibrils increases in tendons with the ageing process [9].

In our experiment comparative examinations of the ultrastructure of tendon tissue exposed to massage in the form of rubbing technique for 60 days and non-massaged (control) group demonstrated statistically significant differences in the thickness and in

number of collagen fibrils. These differences consisted of the decrease of the mean diameter of collagen fibrils and their mean area of transverse cross-section with simultaneous increase of their number in tendons exposed to massage. The results obtained in this study seem to be beneficial for the tendon's tissue physiology. Long-lasting massage (60 days) resulted in the reduction of fibril diameter which is characteristic for tendon tissue in young rats [9]. Oakes (1981; 1988) obtained similar results on ligaments of young rats subjected to exercises in the form of swimming or running in a treadmill [9]. They demonstrated a decrease of the mean diameter of collagen fibrils in the group subjected to exercises as compared to the control group. Furthermore, ultrastructural results of mouse tendons obtained by Michna (cited by [9]) were similar. However, our study cannot give an explicit answer concerning the mechanism causing the observed changes. Based on the structure and function of tendon tissue, it can be assumed that due to the activity of deforming forces that takes place in the course of rubbing technique the deformation of collagen fibrils could occur only in the 'toe' region which represents the load-strain region curve for tendon ('physiological strain range') [9, 12, 13]. Therefore, we suggest that the rubbing technique does not cause injuries both at the macroscopic or ultrastructural level, but it can have a beneficial effect on metabolic activity of fibroblasts [12–14]. These cells synthetize many components of the dense tissue extracellular matrix, especially tropocollagen and glycoaminoglycans, essential for the formation of new collagen fibrils and affecting their numbers. The final effect of massage-induced increased number of collagen fibrils of smaller diameter, which may reflect the higher metabolic activity of tendon's fibroblasts, could signal better adaptation of the tendon to repeated additional lateral deformations which occur in the course of its massage.

To recognize more precisely the mechanisms which act in the connective tissue under the effect of massage, it is essential to perform detailed biochemical and immunocytochemical investigations which would enable determination of changes in the distribution and activity of cells and extracellular matrix of the connective tissue. However, we can conclude that long-lasting repeated massage causes an increase in the number and decrease in the diameter of collagen fibrils in tendon's connective tissue. It can be also presumed that these changes may have a beneficial effect on the metabolic activity of tendon's fibroblasts and in consequence may be important for more effective use of massage for the prevention of tendons' injury and also after the injury has occurred.

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