



Influence of burst TENS stimulation on collagen formation after Achilles tendon suture in man. A histological evaluation with Movat's pentachrome stain

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Retrograde or antidromic stimulation of the nociceptive C fibres is known to lead to the release of sensory neuropeptides, such as substance P (SP), by the peripheral endings of sensory unmyelinated C nerve fibres. These neuropeptides play a role in the healing of soft tissues. Burst TENS (Transcutaneous Electric Nerve Stimulation) is known to be most effective in influencing retrograde C fibre-evoked activity. This is why burst TENS was used in a randomised study as a stimulus for the healing of the sutured Achilles tendon in 9 patients, versus 9 others who received no stimulus. Originally, each group consisted of 10 patients, but there was a single drop-out in each group. Six weeks after surgery a needle biopsy sample was obtained, and stained with Movat's pentachrome stain. It showed a statistically significant influence of burst TENS on new collagen production, maturation of newly formed collagen and organisation of collagen. This suggests that burst TENS might positively influence healing of Achilles tendon suture in man.

INTRODUCTION

Studies during the last century have demonstrated the dual role of the sensitive C-nerve fibres :

1. Sending orthodromic information to the central nervous system, signalling nociception,
2. Triggering neurogenic inflammation antidromically (4, 19) : the neuropeptides, secreted by the sensory nerve terminals, appear to mediate the

interaction between these terminals and the pro-inflammatory cytokines (interleukins, tumour necrosis factor alpha and nerve growth factor) (15).

This explains why an injury to the Achilles tendon induces a neurogenic inflammation which is characterised by oedema formation, increased blood flow, inflammatory cell mobilisation, and release of neuropeptides, such as substance P (SP), calcitonin gene-related peptide (CGRP) and neuropeptide Y (2, 6, 7, 8, 10, 11, 14, 17, 18).

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Ackermann (1) studied the role of SP and CGRP in the healing of the ruptured Achilles tendon in the rat. He noted that peptidergic nerves are absent in the normal Achilles tendon, but present in the paratenon. After rupture of the tendon, nerve fibres grow into the tendon from the surrounding paratenon, as evidenced by the appearance of Growth Associated Protein and Protein Gene Product. This neural ingrowth is soon followed by a striking increase of SP and CGRP neuropeptides in the tendon mass, reaching a peak after the fourth and the second week, respectively. These neuropeptides, visualised by means of immunofluorescence, are typically surrounded by fibroblasts and vessels, which underlines their probable role in the healing process.

The present study was set up to investigate the influence of burst TENS on the C fibres of the tibial nerve after suture of the Achilles tendon in man. Burst TENS was used, because this stimulus is known to selectively affect C fibre activity (16). The authors hoped that the local secretion of neuropeptides at the nerve terminals would increase by post-operative application of burst TENS. By analogy with other studies (5, 9, 20, 21) they expected that increased peripheral release of SP and CGRP would promote healing of the Achilles tendon. In 2003 the authors already demonstrated a statistically significant effect ($p = 0.007$) of burst TENS on fibroblast proliferation in sutured Achilles tendons (3).

PATIENTS AND METHODS

Between June 2002 and June 2003 an acute rupture of the Achilles tendon was sutured in 20 patients. Sports were at the origin in all cases : volleyball in 6 cases, tennis in 6, squash in 3, soccer in 2, badminton, surfing and sprint in 1 each. There were 5 women and 15 men. Their median age was 39 years (range : 27 to 58). All patients were operated on within 24 hours, except four who were treated between 24 and 47 hours postinjury. A lateral approach was used. An open biopsy was performed. The paratenon was incised and the tendon fragments were approximated with a Kessler suture (Ethibond®, 2/0). The frayed ends were sutured with Vicryl®, 4/0. A plaster splint was applied for one week. Subsequently a Bled Shoe® brace was prescribed for 11 weeks, in 10° of plantar flexion during the first 5 weeks. Fraxiparin®

0.3 ml daily was used in all patients. The stitches were removed after two weeks.

Patients were randomised according to their serial number : odd numbers received burst TENS for 30 minutes, five days a week, during the second and third postoperative week ; even numbers did not. The second and third week were chosen because Ackermann (1) noted that SP and CGRP reach a very high level in that period after Achilles tendon section in the animal experiment. No stratification according to sex, nicotine use etc... was performed. An Endomed 982® (Enraf Nonius) TENS apparatus was used ; the electrodes were placed on the calf, above and below the wound. Pulse train duration was 300 ms, internal frequency 100 Hz ; burst frequency was set at 2 Hz. The intensity was as high as tolerable, but without provoking muscle contractions. Pain adjustment resulted in the intensity of stimulation being variable ; it was carefully recorded, every day, as well as the exact duration of treatment.

A needle biopsy was done after 6 weeks in all 20 patients, except in two who dropped out because of re-rupture after two weeks and noncompliance, respectively. A total of 18 patients, 9 in each group, remained available to follow-up. The localisation of the knot of the Kessler suture, and thus of the former gap, was determined ultrasonographically. The skin was marked at this level. After prepping and draping, the area was locally anaesthetised with Lidocain® 2%, containing 1:100.000 epinephrin. A Trucut® biopsy needle was introduced longitudinally, so as to obtain a cylindrical sample, 1 cm long and 1.2 mm in diameter, from the central axis of the tendon, according to the technique described by Movin (13). Specimens were immediately fixed in buffered formalin saline (pH 7.4), dehydrated, and embedded in paraffin wax. Coronal sections were cut at 5 mm thickness and stained with Movat's pentachrome stain (12), allowing to evaluate collagen formation.

The Movat-stained sections were studied at a $\times 400$ magnification (Olympus®-UPIan Apo, oil iris). A ProgRes C14 camera (Jenoptek®, Germany) was mounted on the microscope (Leica®, Germany) and digital pictures were made at 5 random locations along the axis of the sample, from proximally to distally. Histological assessment was carried out at sight to estimate a) the area covered by the newly formed collagen, b) the maturation of the newly produced fibrils and c) the collagen fibril organisation of the healing Achilles tendon.

a) Newly formed collagen density was graded into scale values (table I) : no collagen deposition : value 0 ;

Table I. — Mean area coverage, in percent, by newly formed collagen, per field and per patient, in order of increasing coverage : Assessed by sight on five randomly chosen microscopic fields per patient. Tendency towards more new collagen formation in burst-TENS treated patients ($p = 0.001$)

9 patients treated with burst TENS	9 patients treated without burst TENS
16 (± 5.48)	8 (± 2.74)
17 (± 5.70)	9 (± 6.52)
18 (± 7.58)	10 (± 3.54)
20 (± 10.61)	14 (± 6.52)
22 (± 10.95)	18 (± 3.54)
24 (± 4.47)	18 (± 4.47)
25 (± 6.12)	22 (± 10.37)
26 (± 4.18)	23 (± 5.70)
27 (± 2.74)	26 (± 4.18)
Mean 22.1 (± 6.43)	16.4 (± 5.29)

deposition from 0% to 10% of the area : value 10 ; deposition from 10 to 20% of the area : value 20 ; etc. The mean area coverage, in percent, by newly formed collagen, was assessed by sight on five randomly chosen fields per patient.

- b) The maturation of the newly formed collagen (fig 1) was assessed according to the colour. Recently secreted collagen is dark greenish (collagen maturity value 1), and subsequently turns green - ochre (collagen maturity value 2). As maturation proceeds, it turns ochre (collagen maturity value 3) and then pink-ochre (collagen maturity value 4). Fully matured collagen is red in colour (collagen maturity value 5). These maturation values were estimated by sight on five randomly chosen microscopic fields per patient.
- c) Organisation of collagen deposition (fig 2) was graded into 5 categories : disorganisation, fibres in all directions (organisation value 1), organisation of up to 25% of newly formed collagen fibres (organisation value 2), of up to 50% of new fibres (organisation value 3), of up to 75% of new fibres (organisation value 4), all newly formed collagen fibres in one direction (organisation value 5). These organisation values were estimated by sight on five randomly chosen microscopic fields per patient.

Two blinded independent observers (A.S. and R. F.) evaluated the collagen deposition on the printouts. A Mann-Whitney U (MWU) test was used for statistical computation. Interobserver reliability was examined using the intraclass correlation coefficient.

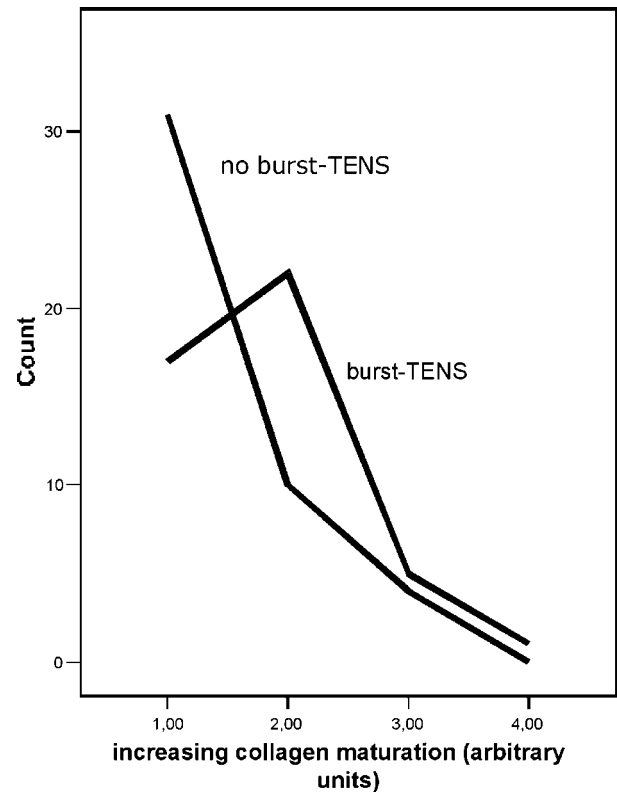


Fig. 1. — Frequency distribution curves of the maturation values of newly formed collagen, assessed by sight on five randomly chosen microscopic fields per patient, according to a 1 to 5 scale, indicating increasing collagen maturation. Continuous line : 9 patients treated without burst TENS. Dotted line : 9 patients treated with burst TENS, showing a tendency towards increasing collagen maturation ($p = 0.006$).

RESULTS

There were two drop-outs (one patient in each group) : one because of re-rupture after 2 weeks, and one because of noncompliance. Consequently, 18 out of 20 patients completed the study. The per-operative biopsy showed localised degenerative lesions in all initial 20 cases, without any sign of metabolic disease. The average intensity of the burst TENS stimulation was 38 ma (SD 19.9).

The average deposition per area of newly formed collagen per patient is expressed in table I : it reached on an average 22.1% (SD 6.43) of the area in "burst TENS" patients, and 16.4% (SD 5.29) of the area in "no burst TENS" patients ($p = 0.001$).

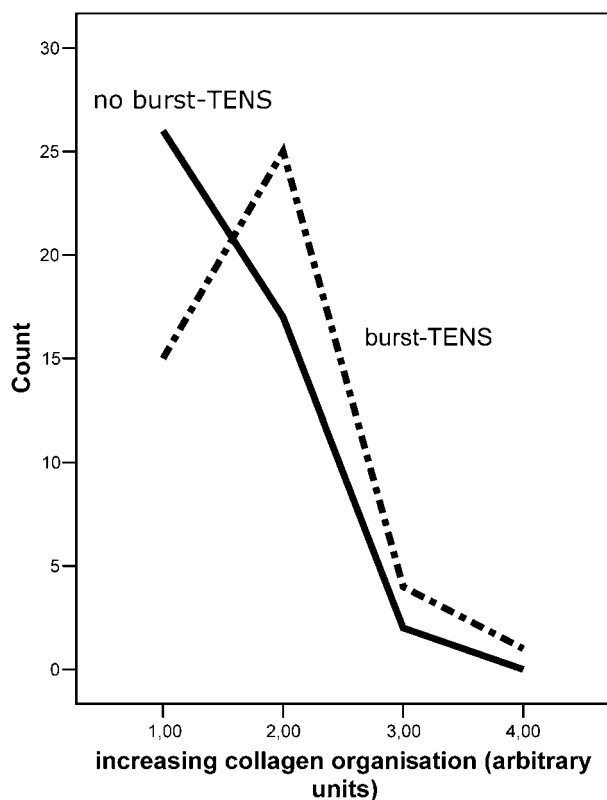


Fig. 2. — Frequency distribution curves of the collagen organisation values, assessed by sight on five randomly chosen microscopic fields per patient, according to a 1 to 5 scale, indicating increasing collagen organisation. Continuous line : 9 patients treated without burst TENS. Dotted line : 9 patients treated with burst TENS, showing a tendency towards increasing collagen organisation ($p = 0.017$).

The frequency distribution of both groups is illustrated in figure 1 for maturation of newly formed collagen and in figure 2 for collagen fibril organisation : the burst TENS-stimulated patients were significantly faster as to maturation ($p = 0.006$) and as to organisation ($p = 0.017$).

Interobserver reliability between the two blinded investigators was 0.785.

DISCUSSION

The purpose of this study was to test the hypothesis that stimulation of the C fibres of the tibial nerve by means of burst TENS would promote healing of the ruptured and sutured Achilles tendon

in man. This would fit into the statement, formulated by laboratory workers, that antidromic stimulation of the nociceptive C fibres forces their peripheral endings to secrete e.g. SP and CGRP, which play a role in tissue healing.

The stimulated group reached a higher level of collagen production as compared with the non-stimulated group, and the difference was significant for collagen deposition per area ($p = 0.001$), maturation ($p = 0.006$) and collagen fibril organisation ($p = 0.017$). Accepting collagen formation as an expression of healing tendency, one would be inclined to consider this result as pleading for the proposed hypothesis. Of course, the better result in the stimulated group might be due to some other mechanism than the release of SP and CGRP. To obtain a better understanding of the role of neuropeptides in tendon healing in humans, further study is mandatory. An immunohistochemical study with antibodies to SP and NK-1 receptor is planned to explore this mechanism.

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