# Influence of burst TENS stimulation on the healing of Achilles tendon suture in man

Peter BURSSENS, Ramses FORSYTH, Adelheid STEYAERT, Edwin VAN OVOST, Marleen PRAET, René VERDONK

**Retrograde or antidromic stimulation of the nociceptive C fibres is known to lead to the release of sensory neuropeptides Substance P (SP) and Calcitonin Gene-Related Peptide (CGRP) by the peripheral endings of these ultra-thin nerve fibres. These neuropeptides have, among others, a vasodilatory effect, which explains why they play a role in the healing of soft tissues. Burst TENS (Transcutaneous Electric Nerve Stimulation) is known to be most effective in influencing 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 10 patients, versus 10 others who received no stimulus. There was one drop-out in each group, so that $2 \times 9$ patients remained available for the study. A needle biopsy, performed after six weeks, showed no significant influence of burst TENS on the histological healing stage, as compared with a rat study. However, a semi-quantitative evaluation of the number of fibroblasts showed a significant advantage for the stimulated group : $p = 0.007$. This means that burst TENS might influence healing of Achilles tendon sutures in man. But above all, it means that a histochemical study of the influence of burst TENS on the release of substance P and CGRP, after suture of the Achilles tendon in man, would be worthwhile.**

## INTRODUCTION

Stricker in 1876 (21) and Bayliss in 1901 (2) were the first to establish that antidromic stimulation of cutaneous sensory nerves leads to cutaneous vasodilation. Hinsey and Gasser (8) found in 1930 that this occurs only when the stimulus is strong enough to excite unmyelinated C fibres. Kenins (11) proved in 1981 that only C fibres of the nociceptive type are responsible for this axon reflex. Celander and Folkow (3) hypothesised in 1953 that this antidromic stimulation of C fibres also occurs in daily life. In their eyes a painful stimulus elicits action potentials in the C fibres that travel not only centrally to signal pain, but also antidromically via a bifurcation of these fibres, so that cutaneous blood vessels are urged to increase their calibre. This so-called axon reflex was considered to be responsible for the "neurogenic inflammation" which follows an external insult, initiating the healing response.

Lembeck (13) hypothesised in 1953 that this kind of vasodilation is mediated by a neurotransmitter, substance P (SP), released from the peripheral endings of sensory nerves. This was confirmed in the inferior alveolar nerve by Olgart *et al* (17) in

*From the Department of Orthopaedic Surgery and Physiotherapy and the Department of Pathology, University Hospital Gent, Belgium.*

Peter Burssens, M.D., Professor.

Adelheid Steyaert, M.D., Physical Medicine and Rehabilitation.

Edwin Van Ovost M.D., Orthopaedic surgeon.

René Verdonk, Professor and Chairman.

*Department of Orthopaedic Surgery and Physiotherapy.*

Ramses Forsyth, M.D. Pathologist.

Marleen Praet, Professor and Chairman.

*Department of Pathology.*

Correspondence : Peter Burssens, Department of Orthopaedic Surgery, University, Hospital, De Pintelaan 185, B-9000 Gent, Belgium.

E-mail : peter.burssens@pandora.be.

1977. Further evidence came from the fact that pretreatment of laboratory animals with capsaicin not only destroys the C fibres but also eliminates antidromic vasodilation and reduces the neuropeptide SP level in sensory neurons (14).

In the last years it has become clear that calcitonin gene-related peptide (CGRP) coexists with and potentiates the effect of SP (22). Their combined appearance in mouse skin flaps was demonstrated by Karanth *et al* (10), and in small dermal axons by Zhang *et al* (23).

In short, nociceptive C fibres have a double task : signalling pain to the central neural system, and conducting an axon reflex, antidromically, towards peripheral nerve endings which are urged to secrete neuropeptides like SP and CGRP. These neuropeptides have a vasodilatory and consequently a healing effect (5, 12).

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 testified by the appearance of Growth Associated Protein (GAP) and Protein Gene Product (PGP). 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. Astonishingly, but not illogically, nerve ingrowth, GAP, PGP, SP and CGRP disappear towards the 16$^{th}$ week after rupture, when the healing process is over.

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 Transcutaneous Electric Nerve Stimulation (TENS) was used, as this stimulus is known to selectively affect C fibre activity (20). The authors hoped that the local secretion of neuropeptides at the nerve terminals would increase by postoperative application of burst TENS. By analogy with other studies (7, 24, 25) they expected that increased peripheral release of SP and CGRP would promote formation of fibroblasts and healing of the Achilles tendon.

## 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 : 6 ; tennis : 6 ; squash : 3 ; socker : 2 ; badminton : 1 ; surfing : 1 ; sprint : 1. There were 5 women and 15 men. Their median age was 39 years (range 27-58). All patients were operated upon within 24 hours, except four who were treated between 24 and 47 hours after trauma. 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 during 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 Ackerman (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, respectively 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 two who dropped out because of re-rupture after two weeks and undisciplined behaviour, 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 anesthetised with Lidocain® 2%, containing

1:100.000 epinephrin. A Trucut, biopsy needle was brought in 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 (15). Specimens were immediately fixed in buffered formalin saline (pH 7.4), dehydrated, and embedded in parafin wax. Coronal sections were cut at 5 mm thickness and stained with hematoxylin and eosin.

The hematoxylin-eosin stained sections were studied at a × 10 magnification (Olympus®-UPIan) for determination of the stage of healing, as compared with the healing stages in the rat Achilles tendon, described by Gigante *et al* (6). Histological assessment was carried out to evaluate the cellularity, vascularity, extracellular matrix collagen density and collagen fibril organisation of the healing Achilles tendon. To quantify the healing stage (expressed in days after suture), sections were examined by one blinded investigator (R.F.) and compared with those obtained in rats by Gigante *et al* (6) at 2, 4, 7, 14, 21, 30, 45 and 60 days after tenotomy of the Achilles tendon. Subsequently it was recorded whether histological healing in the patient corresponded with, lagged behind or was ahead of that in the rat.

Experience taught that the various processes of the healing stage (e.g. vascularity ahead of cellularity) cannot be pinpointed on one day. Therefore observations were classified in an interval scale of a few days (e.g. a healing stage between the 7th and 14th day). However, for statistical analysis, this interval scale was recoded as the mean of these two days (in this case 10.5 days) ; a Mann-Whitney U (MWU) test was used.

The sections were also studied at a × 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 3 locations : the proximal, middle and distal part of the specimen. Two blinded independent observers (A.S. and R. F.) counted the fibroblasts on the printouts. A Mann-Whitney U (MWU) test was used for statistical computation. Inter-observer variability was expressed with the Spearman correlation coefficient.

## RESULTS

There were two drop-outs (one patient in each group) : one because of re-rupture after 2 weeks, and one because of undisciplined behaviour. So 18 out of 20 patients completed the study.

Table I. — Average number of fibroblasts per field and per patient

| Patient | Burst TENS treatment | immobilised |
|---|---|---|
| 1+1' | 131 | 79.3 |
| 2+2' | 170.3 | 131.3 |
| 3+3' | 159.3 | 115 |
| 4+4' | 223.7 | 126 |
| 5+5' | 165 | 77.7 |
| 6+6' | 245.3 | 51.7 |
| 7+7' | 181.3 | 160 |
| 8+8' | 80.7 | 139.7 |
| 9+9' | 131.1 | 127.7 |
| Mean | **165.3** | **112* (P = 0.007)** |

The peroperative biopsy showed localised degenerative lesions in all 20 initial cases, without any sign of metabolic disease. The average intensity of the burst TENS stimulation was 38 ma (S.D. 19.9).

The healing stage, as compared with the images obtained in a rat study by Gigante *et al* (6), averaged 22 days (S.D. 18) in the stimulated group, and 27 days (S.D. 21) in the non-stimulated group ; the difference was not statistically significant (p = 0.39) according to the Mann-Whitney U test.

Fibroblast count after the 6th postoperative week revealed that the median value of the number of fibroblasts per field was 107 (average 112, range 21-165, standard deviation 35) in the non-stimulated group, and 177.5 (average 165.3, range 100-318, standard deviation 49.5) in the stimulated group. This difference is statistically significant (p = 0.007) according to the Mann-Whitney U test**.** The Spearman correlation between assessors was 0.922, i.e. very high. The average number of fibroblasts per microscopic field and per patient is expressed in table I. Average values and S.D. of both groups are illustrated in fig 1.

No correlation could be found between patients' age and fibroblast proliferation.

## 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

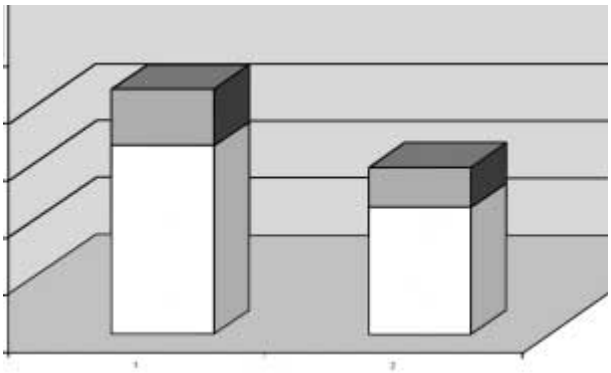

***Fig. 1.*** — Mean number of fibroblasts/microscopic field, and standard deviation. Left (1) : stimulated with burst TENS ; average : 165.3 ; S.D. : 49.5. Right (2) : not stimulated ; average 112 ; S.D. : 35.

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, among others, SP and CGRP, which play a role in tissue healing.

The stimulated group reached no higher level of healing, as compared with a temporally staged study in the rat (6), and the difference with the non-stimulated group was not significant ($p = 0.39$). Extending the study to a larger population might clarify this.

The number of fibroblasts per microscopic field was significantly higher in the stimulated group : $p = 0.007$. Accepting the number of fibroblasts as an expression of healing tendency, one would be inclined to see 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. In this context it is worth mentioning that ultrasound therapy (9), shock wave therapy (18), laser therapy (19), electromagnetic fields (4) and direct-current electrical stimulation (16) have led to similar results, either in man or in the animal model, although it is possible that they all act via the release of SP and CGRP. A histochemical study is planned to explore this question in depth.

*Acknowledgments*

The expert assistance of Michel Hoogmartens was gratefully appreciated.

## REFERENCES

1. **Ackermann P.** *Peptidergic Innervation of Periarticular Tissue. A Study in the Rat.* Thesis, Karolinska Institute, Stockholm, 2001 ; 1-70.
2. **Bayliss WM.** On the origin from the spinal cord of the vasodilator fibres of the hind limb and on the nature of these fibres. *J Phyiol (Lond)* 1901 ; 26 : 173-209.
3. **Celander O, Folkow B.** The nature and distribution of afferent fibres provided with the axon reflex arrangement. *Acta Physiol Scand* 1953 ; 29 : 359-370.
4. **Frank C, Schachar N, Dittrich D, Shrive N, Dehaas W, Edwards G.** Electromagnetic stimulation of ligament healing in rabbits. *Clin Orthop* 1983 ; 175 : 263-272.
5. **Gibran NS, JangYC, Isik FF** *et al.* Diminished neuropeptide levels contribute to the impaired cutaneous healing response associated with diabetes mellitus. *J Surg Res* 2002 ; 108 : 122-128.
6. **Gigante A, Specchia N, Rapali S, Ventura A, de Palma L**. Fibrillogenesis in tendon healing : an experimental study. *Boll Soc Ital Biol Sper,* 1996 ; 72 : 203-211.
7. **Haegerstrand A, Dalsgaard CJ, Jonzon B, Larsson O, Nilsson J.** Calcitonin gene-related peptide stimulates proliferation of human endothelial cells. *Proc Nat Acad Sci USA* 1990 ; 87 : 3299-3303.
8. **Hinsey JC, Gasser HS.** The component of the dorsal root mediating vasodilatation and the Sherrington contracture. *Am J Physiol* 1930 ; 92 : 679-689.
9. **Jackson BA, Schwane JA, Starcher BC.** Effect of ultrasound therapy on the repair of Achilles tendon injuries in rats. *Med Sci Sports Exerc* 1991 ; 23 : 171-176.
10. **Karanth SS, Dhital S, Springall DR, Polak JM.** Reinnervation and neuropeptides in mouse skin flaps. *J Auton Nerv Syst* 1990 ; 31 : 127-134.
11. **Kenins P.** Identification of the unmyelinated sensory nerves which evoke plasma extravasation in response to antidromic stimulation. *Neurosci Lett* 1981 ; 25 : 137-141.
12. **Khalil Z, Helme R.** Sensory peptides as neuromodulators of wound healing in aged rats. *J Gerontol A Biol Sci Med Sci* 1996 ; 51 : B354-361.
13. **Lembeck F.** Zur Frage der Zentralen Uebertragung afferenter Impulse. III. Mitteilung. Das Vorkommen und die Bedeutung der Substanz P in den dorsalen Wurzeln des Rückenmarks. *Naunyn Schmiedebergs Arch Pharmacol* 1953 ; 219 : 197-213.
14. **Lembeck F, Holzer P.** Substance P as neurogenic mediator of antidromic vasodilation and neurogenic plasma extravasation. *Naunyn Schmiedebergs Arch Pharmacol* 1979 ; 310 : 175-183.
15. **Movin T.** Tendon tissue sampling. *Scand J Med Sci Sports* 2000 ; 10 : 368-371.
16. **Nessler JP, Mass DP.** Direct-current electrical stimulation of tendon healing in vitro. *Clin Orthop* 1987 ; 217 : 303-312.

**17. Olgart L, Hökfelt T, Nilsson G, Pernow B.** Localization of substance P-like immunoreactivity in nerves in the tooth pulp. *Pain* 1977 ; 4 : 153-159.

**18. Orhan Z, Alper M, Akman Y, Yavuz O, Yalçiner A.** An experimental study on the application of extracorporeal shock waves in the treatment of tendon injuries : preliminary report. *J Orthop Sci* 2001 ; 6 : 566-570.

**19. Reddy GK, Stehno-Bittel L, Enwemeka CS.** Laser photostimulation of collagen production in healing rabbit Achilles tendons. *Lasers Surg Med* 1998 ; 22 : 281-287.

**20. Sjölund BH.** Peripheral nerve stimulation suppression of C-fiber-evoked flexion reflex in rats. Part 2 : Parameters of low-rate train stimulation of skin and muscle afferent nerves. *J Neurosurg* 1988 ; 68 : 279-283 .

**21. Stricker S.** Untersuchungen über die Gefässwurzeln des Ischiadicus. *Sitz-Ber Kaiserl Akad Wissensch (Wien)* 1876 ; 3 : 173-185.

**22. Woolf C, Wiesenfeld-Hallin Z.** Substance P and calcitonin gene-related peptide synergistically modulate the gain of the nociceptor flexor withdrawal reflex in the rat. *Neurosci Lett* 1986 ; 66 : 226-230.

**23. Zhang J, Huang W, Tuckett P.** C-fiber modulation of the rat type I slowly adapting mechanoreceptor. *Neuroscience* 2002 ; 115 : 797-804.

**24. Ziche M, Morbidelli L, Pacini M, Dolara P, Maggi CA.** NK1-receptors mediate the proliferative response of human fibroblasts to tachykinins. *Br J Pharmacol* 1990 ; 100 : 11-14.

**25. Ziche M, Morbidelli L, Pacini M, Geppetti P, Alessandri G, Maggi CA.** Substance P stimulates neovascularization in vivo and proliferation of cultered endothelial cells. *Microvasc Res* 1990 ; 40 : 264-278.