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ABSTRACT. *Background:* Pharmacological treatment for diabetic polyneuropathy (DP) has shown limited benefit; frequency-modulated electrical stimulation (FREMS) has shown positive results in pain control and nerve conduction velocity in DP. *Objective:* To investigate the effects of FREMS vs transcutaneous electrical nerve stimulation (TENS) on the release of vascular endothelial growth factor (VEGF) in Type 2 diabetic and in non-diabetic subjects. *Methods:* 10 non-diabetic [mean age 37±5 yr; females (F)/males (M): 6/4] and 10 Type 2 diabetic subjects (mean age 52±6 yr; F/M: 5/5) with DP underwent TENS (for 10 min) followed by 30 min interval without electrical stimulation, and then FREMS (for 10 min) over the forearm volar surface. Blood samples for VEGF measure-

ments were obtained from the contra-lateral arm every 2 min during TENS/FREMS application and every 10 min during the intervals. *Results:* We observed a significant rise in plasma VEGF during FREMS in both non-diabetic and diabetic subjects (maximal response 89.4±80.3 pg/ml and 48.5±18.3 pg/ml, respectively; $p<0.01$ vs basal) with a lower, but still significant response in diabetics. No changes in VEGF were observed during TENS application. *Conclusion:* VEGF release during FREMS may help explain the positive effects on nerve conduction velocity in DP, possibly mediated by favorable effects on *vasa nervorum* microangiopathy.

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INTRODUCTION

Peripheral neuropathy is a frequent complication of long-term diabetes that ultimately accounts for considerably disabling morbidity (1, 2). Clinical hallmarks of diabetic polyneuropathy (DP) are chronic pain and impaired nerve conduction velocity in both, motor and sensory pathways, while histopathological features include *vasa nervorum* microangiopathy, and axons atrophy/loss resulting from different tissue damage mechanisms common to all long-term diabetic complications (1). Final consequences of such damage include foot ulcerations, accounting for ~20% of hospital admissions among diabetics (3), which may lead to lower ex-

tremity amputation, three times more common in diabetic compared to non-diabetic subjects (4). Proposed pathogenetic mechanisms for DP include metabolic injury to nerve fibers (5), *vasa nervorum* damage (6), sorbitol accumulation (7), fatty acids alterations (8), protein glycosylation (9), oxidative stress with changes in NAD:NADH ratio (10), neurotrophic growth factors reduction (11), and autoimmune damage (12). This multifactorial process lead to progressive nervous fiber demyelination, functional deterioration and suppressed regeneration (1, 2). Gene transfer of plasmid DNA encoding vascular endothelial growth factor (VEGF) has been studied in experimental diabetic animals with positive results (13). Induction of VEGF synthesis and release by sub-contraction electrical stimulation in skeletal muscle cells *in vitro* and in experimental hind limb ischemia *in vivo* has been reported (14). Current evidence have demonstrated the central role that glycemic tight control and reduction of other cardiovascular risk factors may have on DP development and progression (2), but not in the modification of the disease once established. Phar-

Key-words: Diabetic neuropathy, diabetes mellitus, VEGF, electrical stimulation, TENS.

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macological trials for DP have shown limited benefit, hence, some attention has been given to non-pharmacological approaches. Transcutaneous electrical nerve stimulation (TENS) showed benefit in pain relief (15) and, recently, electrical transcutaneous stimulation with modulated frequencies (FREMS) has demonstrated positive effects not only on pain control, but also on nerve conduction velocity (16). The present study aimed to investigate the effects of FREMS compared to TENS on VEGF release in diabetic and non-diabetic subjects.

SUBJECTS AND METHODS

Ten diabetic patients [mean age 52 ± 6 yr; females (F)/males (M): 5/5] with DP were recruited from the Diabetes Outpatient Clinic and 10 nondiabetic subjects (mean age 37 ± 5 yr; F/M: 6/4) were recruited from our department's employees who volunteered to participate in the study after being carefully informed about the purpose and the procedures involved. All participants gave informed consent, the study was approved by the local Ethics Committee and conducted in accordance with the Declaration of Helsinki. All subjects underwent TENS for 10 min, followed by an interval of 30 min without electrical stimulation. Then FREMS therapy was applied for 10 min. Both electrotherapies were administered with APTIVA neurostimulator (Lorenz Therapy™ System, Medolla, MO, Italy) via 2 non-woven electrodes applied over the forearm volar surface. DP was identified with electromyography (Medelec™ Synergy N-EP-EMG/EP Monitoring System). According to international criteria and standardization at our institution, two of the following criteria, bilaterally, were considered for DP diagnosis (mean of 6 speed conduction for each nerve): 1) ulnar motor conduction velocity (MCV) < 48 m/sec, sensory conduction velocity (SCV) < 50 m/sec; 2) common peroneal MCV < 45 m/sec and/or amp < 3 mV; 3) posterior tibialis MCV < 42 m/sec and/or amp < 4 mV; 4) suralis SCV < 48 m/sec and/or amp < 8 mV.

FREMS consisted of sequences of mono-phase compensated negative potential electric pulses, with sharp spike and asymmetrical shape; peak amplitude varied from 0 to 255 Volts; pulse frequency varied from 1-50 Hz; pulse duration varied from 10-40 microseconds. Blood samples for plasma VEGF measurements (enzyme-linked immunosorbent assay; R&D Systems) were obtained from the contralateral arm through intravenous indwelling catheter at different time points (indicated in Fig. 1) during 70 min. Statistical analysis was performed using GraphPad software version 4.0 (GraphPad, San Diego, CA, USA), considering statistical significance for p values below 0.05.

RESULTS

Plasma levels of VEGF were obtained before, during, and after both electrostimulation therapies. The mean VEGF concentration \pm SD obtained in non-diabetic subjects (upper panel) and Type 2 diabetic patients with DP (lower panel) are shown in Figure 1. FREMS treatment resulted in maximum VEGF concentrations during the 10 min applica-

tion, followed by a decrease of VEGF concentrations towards the baseline levels in both non-diabetic and diabetic subjects (Fig. 1) ($p < 0.001$ according to analysis of variance for repeated measures). The VEGF elevation observed in correspondence to FREMS administration was not observed during TENS application in both, non-diabetic and diabetic subjects. Indeed, VEGF concentrations during TENS were similar to those observed during the intervals before application of the electrostim-

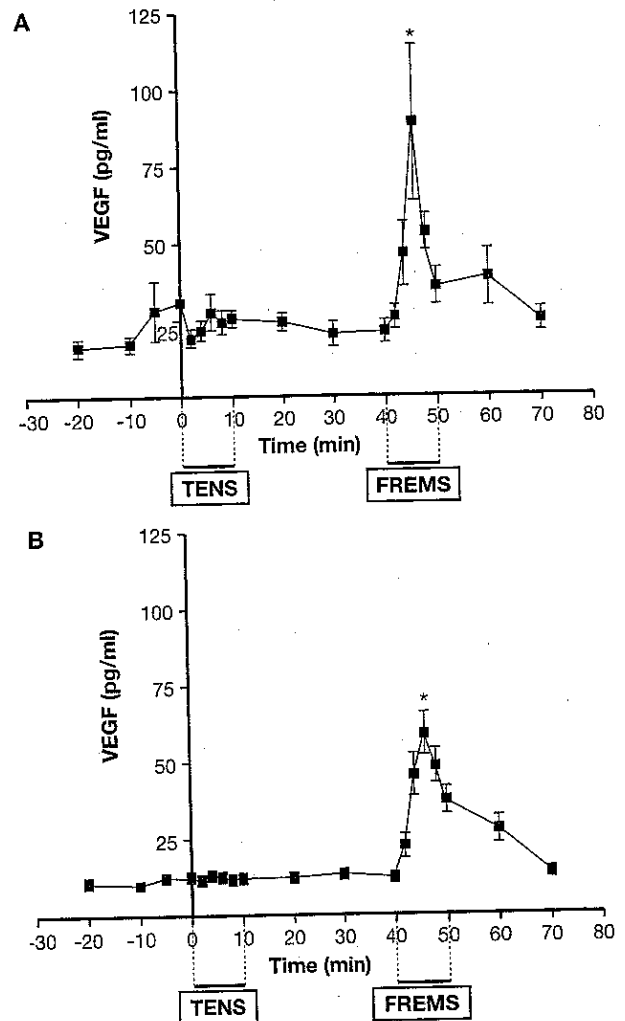


Fig. 1 - Plasma vascular endothelial growth factor (VEGF) levels during (transcutaneous electrical nerve stimulation) TENS (0-10 min) and during frequency-modulated electrical stimulation (FREMS) (40-50 min) administration in non-diabetic subjects (panel A) and in Type 2 diabetic subjects with polyneuropathy (panel B). There was a significant increase of serum VEGF concentration during FREMS application in both groups, with no significant increase in VEGF concentrations during TENS application. *means $p < 0.001$.

ulation therapies. The areas under the curve for VEGF release, calculated with the trapezoidal rule, were as follows: in non-diabetics 490.1 ± 171.8 vs 246.2 ± 98.3 pg/ml during 10 min for FREMS vs TENS application, respectively ($p < 0.01$); in diabetics, 400.6 ± 124.8 vs 122.1 ± 41.0 pg/ml during 10 min for FREMS vs TENS application, respectively ($p < 0.001$). Although maximal responses were lower in diabetics (48.5 ± 18.3 pg/ml; $p < 0.01$ vs basal), compared to the maximal responses in non-diabetic subjects (89.4 ± 80.3 pg/ml; $p < 0.01$ vs basal) they were still present and significant.

DISCUSSION

Despite recent evidence that not only glycemic tight control but also the control of hypertension, overweight, high triglyceride levels, and smoking may influence the development of DP (2), few evidence-based strategies demonstrating improvement or prevention of this devastating condition are available. Controlled pharmacological trials for DP proved heretofore disappointing even with initial encouraging reports. Hence, an urgent need to develop new therapeutical approaches that improve nerve function in diabetic patients is warranted.

Non-pharmacological therapeutical attempts tested up to now include TENS, which showed some benefit in pain reduction (15), but without affecting nerve conduction velocity. We and others have used electro-magnetic neural stimulation with frequency modulation, FREMS, in patients with DP. FREMS therapy induces both, pain relief and significant increases in motor nerve conduction velocity in these patients (16).

An experimental study tested the hypothesis that DP results from *vasa nervorum* destruction and that it can be reversed by administration of angiogenic growth factors. Rats with streptozotocin-induced diabetes and rabbits with alloxan-induced diabetes in whom nerve blood flow and vessel number were markedly attenuated, paralleled to significant slowing of motor and sensory nerve conduction velocities, were compared to control non-diabetic animals. Intramuscular gene transfer of plasmid DNA encoding VEGF-1 or VEGF-2 induced revascularization and blood flow restoration in nerves of treated animals to levels of those observed in non-diabetic controls; constitutive overexpression of both transgenes resulted in restoration of large and small fiber peripheral nerve function (17). These results support the notion that DP results from *vasa nervorum* ischemia, hence, VEGF is proposed as a candidate treatment for this condition.

VEGF, the most potent endogenous angiogenic factor so far identified, is produced in cells around the vasculature and acts selectively on vascular endothelial cells as a paracrine factor stimulating angiogenesis both *in vitro* and *in vivo*. Expression of VEGF is highly regulated by hypoxia, providing a feedback mechanism to improve oxygenation via the promotion of new vessel formation (2). Kanno et al. showed the induction of VEGF protein synthesis and release by sub-contraction electrical stimulation in skeletal muscle cells in culture. This effect was confirmed *in vivo* in an experimental model of hind limb ischemia, in which increased muscle blood flow and VEGF synthesis was observed after electrical stimulation (14).

The difference in VEGF release observed in the present study with two electrotherapies may reside in the variability of electrical stimulus frequency and voltage amplitude induced by FREMS, but not by TENS. The modulation of these parameters induces composite Motor Action Potentials (MAP) in excitable tissues by the summation of sub-threshold electric stimulations, conveyed through the skin over the motor nerve. A single, low-intensity and brief duration impulse, such as the one delivered by TENS, is unable to overcome the dielectric skin barrier and excite the underlying nervous and/or muscle to elicit a recordable MAP; this effect can be achieved by FREMS through specific sequences of weak impulses with rapid increases and decreases in pulse frequency and duration, which may result in gradual recruitment of MAP in the stimulated tissues (18). MAP induction in the underlying neural tissue may have stimulated endothelium of *vasa nervorum* to release VEGF into the circulation. An improvement in endoneural blood flow, hypoxia, and microvessel disease may help to explain the positive reported effects of FREMS on DP (16). In fact, blood flow improvement with either revascularization or physical exercise is accompanied with increased nerve conduction velocity. Studies on the chronic effect of FREMS on VEGF synthesis and release are needed in order to clarify the nature of long-term FREMS actions.

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