International
Journal of Neurorehabilitation
ISSN: 2376-0281

Segal et al., Int J Neurorehabilitation 2016, 3:2
DOI: 10.4172/2376-0281.1000203

Research Article Open Access

The Effect of Electromagnetic Field Treatment on Recovery from Spinal Cord Injury in a Rat Model – Clinical and Imaging Findings

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Rec date: March 01, 2016; **Acc date:** March 21, 2016; **Pub date:** Mar 28, 2016

Abstract

Background: Spinal cord injury (SCI) refers to spinal cord damage arising from trauma, disease or degeneration. At present, there is still no treatment for any paraplegia resulting from SCI. We have previously shown that very low intensity, low frequency, electromagnetic field treatment (VLIFE) promotes neuronal plasticity after stroke and as a result, improves clinical recovery.
Objective: In this paper we studied an innovative electromagnetic field treatment for SCI in an animal model.
Methods: SCI was caused to 20 rats by hemi crush. The animals were divided into three groups, 7 animals were not treated, two groups received VLIFE treatment for two months, 7 rats received 15.72 Hz, and 6 rats received a dedicated treatment of 26 Hz. Clinical evaluation was performed weekly, and imaging assessment monthly.
Results: Clinical performance assessed by a locomotor test, show significant clinical improvement of the neurological function following treatment with VLIFE ($p < 0.05$). Imaging results after two months of treatment, by MRI including DTI analysis, show that the non-treated (sham) spinal cord has not recovered, while in the treated animal the fibers of the spinal cord were preserved and rewired. VLIFE treatment has major benefits on injured spinal cord: preservation of the spinal cord from further degradation caused by the edema and internal cord scars, and rewiring of the spinal cord resulting with rehabilitation and improved clinical performance.
Conclusions: Low intensity low frequency electromagnetic field treatment may be beneficial for rehabilitation from SCI, human clinical trials are planned.

Keywords: Spinal cord injury; Electromagnetic field; Neurogenesis; Rehabilitation; VLIFE

Abbreviations

SCI: Spinal Cord Injury; VLIFE: Very Low Intensity, low Frequency, Electromagnetic field treatment; DTI: Diffusion Tensor Imaging; BBB: Basso, Beattie, Bresnahan Locomotor rating scale

Introduction

SCI is a medically complex and life-disrupting condition. SCI refers to traumatic or non-traumatic damage to the spinal cord. It is usually caused by a physical impact to the spinal cord resulting with damage [1]. The severity of the injury depends on both the strength of the impact and its location. In general, the higher along the spinal cord the impact, the greater the severity of SCI. SCI contributes to about 23% of all causes for paralysis. Most people having SCI will survive the injury and will be released from the hospital in a range of paralysis. Less than 1% of persons experienced complete neurologic recovery by the time they are discharged from the hospital. SCI has costly consequences, for individuals, family and society. People are left dependent and are less likely to be employed. Worst of all, they risk premature death. For a young person who suffered the injury at the age of 25 the expected expenditure for life is about 3 million dollars [1,2].

According to the US Center for Disease Control and Prevention and recent studies, there are about 20,000 new patients having SCI annually in the US and total of about 250,000 patients living with SCI [3]. Among the causes for SCI, motor/vehicle accidents, war/terror/violence injuries, accidents at work and sport/recreation are responsible for most cases.

Age distribution of SCI shows that while the majority of SCI occurs between the ages of 40 to 60, over 20% of SCI occurs below the age of 40, leaving people with SCI to live a whole life in paralysis. At present, while there are several studies being carried out with stem cell therapy and with alternative movement technologies which are being developed, there is still no cure for any paraplegia resulting from SCI [4-7].

We have previously shown that very low intensity, low frequency, electromagnetic field treatment (VLIFE), targeting a specific functional neural network by frequency rather than location, promotes neuronal plasticity after stroke and as a result, improves clinical recovery [8]. In this paper we studied this innovative treatment for SCI in an animal model.

Methods

This study was performed in compliance with "The Israel Animal Welfare Act" and following "The Israel Board for Animal Experiments" approval No. IL-13-10-191.

Spinal cord injury (hemi-crush)

The following procedure was performed on 20 rats.

On completion of the behavioral training, the rats were deeply anesthetized with xylazine (15 mg/kg) and ketamine (70 mg/kg) injected intra-peritoneal. A midline skin incision was made in the mid-thoracic region. The paravertebral muscle was retracted and the lamina of vertebra T9 was carefully removed with a bone crusher. The exposed dura was incised, and the spinal cord was exposed. The midline artery was identified and a crush injury was inflicted for 60 seconds, using jewelers' forceps. Hemi-crush injury was inflicted by crushing the spinal cord along only half of its width. Special care was taken to cause minimal damage to the meninges and to avoid damage to the blood supply by sparing the midline posterior artery. After the spinal cord hemi-crush injury the wound was sutured. To prevent infections, especially bladder infections, the rats were treated immediately after surgery with antibiotics daily for at least 3 days and as needed thereafter, and by mechanical emptying the bladder at least twice a day until spontaneous urination was recovered. In addition, the animals were treated by analgesic (Carprofen 5 mg/kg) administered SC for at least 3 days after the surgery and as needed thereafter [9].

Clinical Evaluation

Basso, Beattie, Bresnahan (BBB) locomotor rating scale

Function recovery was tested according to BBB scale for locomotor assessment after SCI [10]. The scale (0-21) represents sequential recovery stages and categorizes combinations of animal joint movement, hind limb movements, stepping, forelimb and hind limb coordination, trunk position and stability, paw placement and tail position. Results are expressed as change from baseline. The animals were evaluated at days 1, 7, 14, 21, 28, 43, 58 (day 0 is hemi-crush).

VLIFE treatment - very low intensity, low frequency, electromagnetic field treatment

The animals were divided into three groups, Control (M1) which were placed in the VLIFE device (Figure 1) and were not subjected to any electromagnetic field. General treatment (M2) which was given the alternating field that was found to be the most effective to the motoric nervous system in stroke treatment at 15.72 Hz [8] and dedicated treatment (M3) that received the frequency of 26 Hz which was measured in reference to the movement of the lower limb.

Figure 1: VLIFE device for animal treatment.

Treatment was conducted in two session, in the first month, the animals were subjected to 8 min long treatment per day for five days a week, which was found to rehabilitate traumatic brain injury (TBI) in a previous experiment (data on file) and during the second month, duration of treatment was increased to 20 min.

Time points (TP) for clinical and imaging evaluation

TP1- 3 days after hemi crush. TP2 after 1 month of treatment. TP3 - after 2 months of treatment. All animals were sacrificed at day 58.

Imaging - MRI protocols and analysis

MRI protocol

MRI was performed in 7T MRI system, Bruker, Germany using a 20 mm surface coil placed on the back of the rats in the region of injury. Rats were anesthetized using 1-3% isoflurane and maintained in 37°C, their breathing was monitored with a breathing sensor. MRI protocol included the following sequences:

T2 Rapid acquisition refocused echo *(*RARE)

Sagittal T2 weighted images were performed in order to localize the axial slices in the correct location, including upstream and downstream regions adjacent to the injury site. T2 RARE included the following parameters: TR/TE=1200/16, RARE factor=4, no. of averages=4, 20 slices of 0.8 mm, in-plane resolution of 0.17 × 0.2 mm (matrix size 128 × 128 and FOV of 25.6 × 22.8 mm).

DTI

DTI was performed with the following parameters: TR/TE=4500/30 ms, 4 EPI segments, Δ/δ=10/4.5 ms, 15 non-collinear gradient directions with a single b value shell at 1000 sec/mm^2 and one image with b value of 0 sec/mm^2 (referred to as b0), 3 averages, 2 repetitions. Geometrical parameters were: 18 axial slices of 1 mm thickness (brain volume) and in-plane resolution of 0.156 × 0.156 mm^2 (matrix size of 128 × 128 and FOV of 20 mm^2). Duration of one DTI repetition was 14:24 mins.

Quantitative T2

T2 weighted imaging was performed with the following parameters: MSME sequence; TR=2500 ms; 10 different TE (ms):10, 20, 30, 40, 50, 60, 70, 80, 90, 100; 2 averages. Geometrical parameters were: 18 slices of 18 slices of 1 mm thickness and in-plane resolution of 0.078 × 0.078 mm^2 (matrix size of 256 × 128 reconstructed to 256 × 256 and FOV of 20 mm^2). Duration of one MSME repetition was 10:40 mins.

Image Analysis

DTI fiber tracking

DTI calculation and fiber tracking was performed using Explore DTI software [11]. The tensors obtained were spectrally decomposed to their eigen-components. The eigen-values were used to calculate FA and MD maps [12]. Tractography was applied using Deterministic (streamline) fiber tracking, terminating at voxels with FA lower than 0.2 or following tract orientation change higher than 20°. Fibers that passed through a manually chosen seed region of interest (ROI) were plotted. The fibers were plotted as streamlines. The masks obtained

were overlaid over the color-coded FA image. The average FA and MD values were extracted in "injured" and "intact" regions of the spinal cord fiber tract.

Two representative images are presented, one animal of the treated (M3) and one non-treated (M1) representing their groups.

Results

Clinical results were obtained from the animals that have survived the whole length of the experiment and that their hemi-crush resulted in severe damage to the spinal cord on one hand, and left no bone fragment in the spinal cord canal on the other hand, resulting in 3 animals in group M1, 3 animals in group M2 and 4 animals in group M3.

Clinical

Results of the recovered animal function, assessed by BBB locomotor test, show significant clinical improvement of the neurological function following treatment with VLIFE ($p < 0.05$) as shown in Figure 2. While both treatment groups improved, the group which received a dedicated treatment (M3) did not reach its peak of improvement after 57 days.

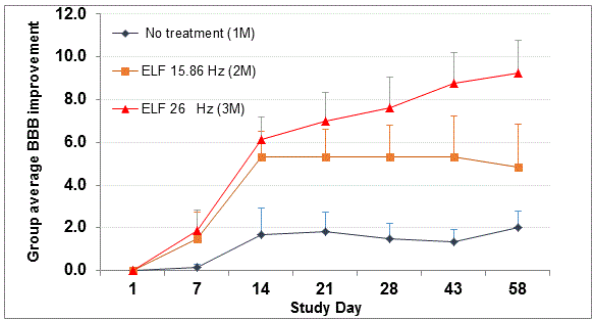

Figure 2: BBB shows clinical result of recovery from lower spinal cord injury. Both VLIFE treatment frequencies show significant recovery (results are mean±SE, difference from baseline, P for M2 and M3 <0.005).

Imaging

In Figure 3 the MRI findings on day 3 are depicted. The location of the hemi-crush is demonstrated, in both spinal cords (treated and non-treated) the extent of edema can be visualized as hyper intense signal in the T2 image.

DTI fiber tracking shows that in the non-treated rat the connectivity is truncated along the spinal cord and the degradation of the spinal cord has begun. In the treated rat, however, where the spinal cord was completely transected, the DTI reveals that even though the edema displaced the spinal cord in the region of the hemi-crush, there is potential connectivity along the spinal cord.

In Figure 4, MRI findings on day 29 are demonstrated. In the non-treated rat, the edema is larger than that presented in the treated spinal cord, while there is no evidence of healthy spinal cord in the region of the hemi-crush. The DTI also shows further degradation of the spinal cord and a larger gap between the remaining upper healthy part of the spinal cord and the lower affected part.

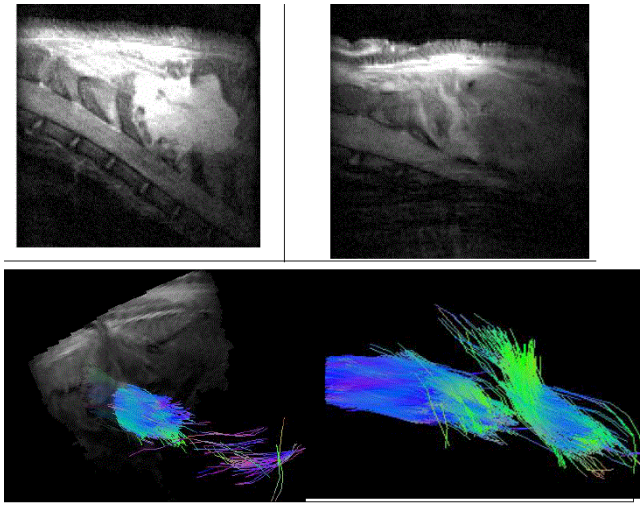

Figure 3: Non-treated spinal cord (left panels) MRI image (upper panel) and DTI fiber tracking (lower panel), treated spinal cord (right panels) MRI image (upper panel) and DTI fiber tracking (lower panel), at day 3.

The treated spinal cord, not only shows that portion of the spinal cord has recovered; it also shows that it has re-connected with the dorsal part of the spinal cord and regained its ability to carry on normal function.

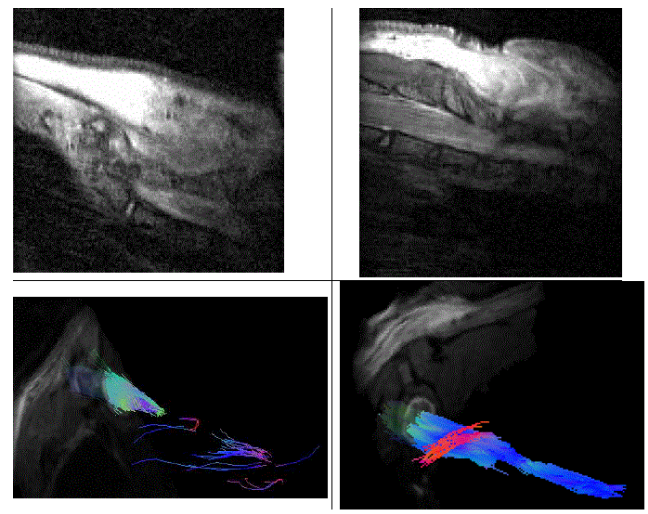

Figure 4: Non-treated spinal cord (left panels) MRI image (upper panel) and DTI fiber tracking (lower panel), treated spinal cord (right panels) MRI image (upper panel) and DTI fiber tracking (lower panel), at day 29.

Figure 5, MRI findings on day 57 are shown, demonstrating that the non-treated spinal cord has not recovered and its condition continued to degenerate with time, which is evident from the DTI (the lower part

of the hemi crush). On the other hand, in the treated rat, it is evident that even if the path of the spinal cord was diverted by the scar on the spinal cord tunnel, and therefore narrowed, a large portion of the spinal fiber tracts successfully re-wired, resulting in regeneration.

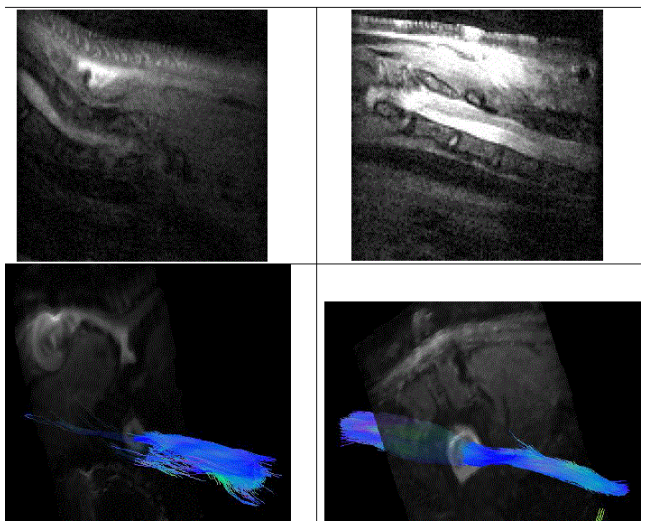

Figure 5: Non-treated spinal cord (left panels) MRI image (upper panel) and DTI fiber tracking (lower panel), treated spinal cord (right panels) MRI image (upper panel) and DTI fiber tracking (lower panel), at day 57.

Discussion

It has been reported that electrical and magnetic trans-spinal stimulation can be used to increase the motor output of multiple spinal segments and modulate cortico-spinal excitability [13]. In this research we have shown that VLIFE, an electromagnetic field treatment, has two major benefits on injured spinal cord. A) preservation of the spinal cord from further degradation caused by the edema and internal cord scars, preserving its potential conductivity even though it was completely transected and displaced, and B) when the treatment is tuned to a specific working operation of a given neural network, the spinal cord following transection was not only preserved from degeneration, fiber tracts were re-wired, resulting in a rehabilitation process, improved clinical performance and regained functions. Thus, suggesting that further clinical recovery may occur in later stages in the rehabilitation process. It is interesting to note though, that spinal cord potential function was preserved even when treatment was not specific, which suggests that a "general treatment" can be applied to SCI patients soon after injury while the clinical condition of the patient is yet unclear.

Our findings show that the internal spinal cord scar limited the space that is available to the re-growth of the spinal cord, thus suggesting that in the case of humans, maybe anti-scar treatment, surgical or other [14] may allow a wider space for the spinal cord to grow in, furthering the possibility of rehabilitation.

An important observation was that the treated rats, which were at baseline paralyzed in their hind limbs, recovered their limb larger muscles functionality but not their fingers; as a result the rat was able to run but not to catch the beam (available on video). Therefore suggesting that for each motoric/sensory neurologic network, a different treatment protocol should be used in order to address the recovery of all relevant networks. The fact that the progress of rehabilitation did not reach its maximum benefit under the dedicated treatment by day 57 emphasizes the importance of the specificity of the treatment and suggests that enhanced rehabilitation by VLIFE may be relevant for acute and chronic time frames.

The presence of voltage gradients within developing and damaged tissues led already in the past to the notion that the resultant electrical fields provide instructional cues to cells. [15]. The effect of the VLIFE treatment may be attributed to these neurogenesis characteristics [8], which possibly involve activation of relevant growth factors and enzymes [16,17], as well as some neuroprotective activity [18]. Among the possible mechanisms which could explain the beneficial effect of an electromagnetic field, the following have been mentioned: neuronal protein synthesis, calcium ion/neurotransmitter effects at synapse, and angiogenesis [19].

A limitation of the current study was the fact that the VLIFE affected only the lower limb larger muscles because of the specific treatment choice. Also the effect did not reach plateau, implicating that the protocol was not optimal. Further treatment protocols, and their possible combinations, should be studied.

The results of our previous research on VLIFE treatment for stroke [8], together with this study results, accentuate the importance and potential of this innovative treatment. Additional clinical trials may further prove its efficacy for humans.

VLIFE device and treatment are patent pending.

Disclosure

Dr. Segal Y, Mr. Segal L, Shoham E, Alter A are employed by BrainQ Ltd. Study was sponsored by BrainQ Ltd.

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