Received: May 31, 2018 Revised: August 1, 2018 Accepted: August 15, 2018

(onlinelibrary.wiley.com) DOI: 10.1111/ner.12866

Selective L4 Dorsal Root Ganglion Stimulation Evokes Pain Relief and Changes of Inflammatory Markers: Part I Profiling of Saliva and Serum Molecular Patterns

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Objectives: Complex regional pain syndrome (CRPS) and associated comorbidities have been linked to a pro-inflammatory state driven by different mediators. Targeted dorsal root ganglion stimulation (DRG_{STIM}) suppressed pain levels and improved functional capacity in intractable CRPS. However, clinical trials assessing the impact of DRG stimulation on the neuroimmune axis are lacking.

Methods: This study enrolled 24 subjects (12 refractory CRPS patients plus suitably matched healthy controls) and performed immunoassays of inflammatory mediators in saliva and serum along with score-based assessments of pain, mood, and sleep quality at baseline and after three months of selective L4-DRG_{STIM}.

Results: After three-month L4-DRG_{STIM} CRPS associated pain significantly decreased. In addition, disturbed sleep and mood improved post-DRG_{STIM}, although statistically not significant. Significantly increased serum values of pro-inflammatory markers were detected pre- and post L4-DRG_{STIM} for high-mobility group box 1, tumor-necrosis factor α, interleukin (IL) 6, and leptin. IL-1β was significantly elevated pre-L4 DRG_{STIM}, but not posttreatment. Elevated anti-inflammatory IL-10 significantly decreased after three months in serum, while saliva oxytocin concentrations increased in CRPS subjects after L4-DRG_{STIM} ($p = 0.65$). No severe implantation and stimulation associated adverse events were recorded.

Conclusions: Selective L4-DRG_{STIM} improved neuropathic pain and functional impairment in CRPS as previously reported. CRPS patients displayed a pro-inflammatory molecular pattern in serum. Serum anti-inflammatory IL-10 significantly declined,

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Source(s) of financial support: Investigator Initiated Trial (ITT) from Abbott (formerly St. Jude Medical, Inc.).

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while saliva oxytocin nonsignificantly increased after L4-DRG_{STIM}. An evidence-based relational interpretation of our study is limited due to the uncontrolled study design. However, molecular profiling of biofluids (saliva, serum) represents a novel and experimental field in applied neuromodulation, which warrant further investigations to unveil mechanisms of neuroimmune modulation.

Keywords: Chronic neuropathic pain, dorsal root ganglion stimulation, immunomodulation, inflammation, quantitative outcome measures

Conflict of Interest: Thomas M. Kinfe has received training support and works as a consultant for Abbott (formerly St. Jude Medical, Inc.) and works as a consultant for Medtronic Inc. Sajjad Muhammad has received a Neuromodulation and Pain Fellowship by Abbott Inc. (formerly St. Jude Medical). Krishnan Chakravarthy is a consultant to Abbott (formerly St. Jude Medical) and Bioness Inc. Jeff Kramer is an employee of Abbott Inc. Thomas L. Yearwood works as a consultant for Abbott (formerly St. Jude Medical, Inc.), Boston Scientific Neuromodulation, Nevro Corp., and Neuronano and is CMO of Meagan Medical. The remaining authors have no conflicts of interest to disclose.

INTRODUCTION

Total knee arthroplasty (TKA) due to osteoarthritis and especially after revisions of previous TKA have been reported to lead to a refractory chronic postsurgical pain (CPSP). The incidence of CPSP ranges between 10 and 34% and additionally impact quality of life, mood, sleep, cognition, and metabolic state of the affected subjects (1,2). Complex regional pain syndrome I–II (CRPS) represent clinical phenotypes of CPSP of the knee region (3,4). In case revision surgery, pharmacological and behavioral therapy fail to achieve a sustained improvement, consideration of adjunctive neuromodulation treatment strategies has been recommended.

Conventional spinal cord stimulation (SCS) suppressed CRPS pain levels by 40–50% in the past. Most recently, an approach that appears to have a considerable promise for treating focal neuropathic pain has become available (namely, dorsal root ganglion stimulation [DRG_{STIM}]). Anatomically targeted DRG_{STIM} was found to be superior to conventional SCS in a Class I study as well as several controlled and uncontrolled observational clinical trials for a variety of pain disorders (5–10). Briefly, DRG_{STIM} may have the capability to restore the distorted filter function of the DRG, thus, inhibiting hyperexcitability of DRG neurons and deeper layer compartments (laminae II/III) of the spinal cord. The precise mechanism of DRG-evoked effects on spino-nociceptive neural transmission as yet is not fully established (11–14). So far, mainly studies with neuropsychiatric measures have addressed the unmet question for possible predictive factors relevant for patient selection and neurostimulation treatment (15,16).

The analgetic potential of oxytocin (OXY) via descending pathways by means of direct GABAergic inhibition of Aδ and C fiber (primary afferent excitation in deeper spinal cord layers) and/or via OXY receptors on nociceptive C fiber afferents of DRG neurons has been documented in several experimental studies (17–24). In addition, preclinical findings indicate a pro-inflammatory state mediated by cytokines/chemokines in chronic neuropathic and nociceptive pain syndromes (25–40). Preliminary open-label human pilot studies observed that the tonic and BurstDR SCS substantially impact CSF and serum concentrations of pro- and anti-inflammatory biomarker pattern in both subtypes of chronic pain (41,42).

Therefore, it seems reasonable to develop additional screening tools in order to improve patient selection and neurostimulation treatment monitoring. The goal of this study was to assess concentration changes of neuroinflammatory mediators in serum (interleukins [IL-1β, IL-6, IL-10], tumor-necrosis factor [TNF-α], high-mobility group box 1 [HMGB-1], brain derived neurotrophic factor [BDNF], leptin, adiponectin, ghrelin) and in saliva (oxytocin; OXY) relative to selective L4-DRG therapy compared to an age/gender matched healthy control group. Secondary goals include score-based assessments of the changes of pain (NRS), functional measures (sleep, mood, metabolic state), and DRG stimulation parameters.

METHODS

This single-center study included patients with chronic refractory neuropathic CPSP, of whom the majority was classified as CRPS I/II. The study protocol received approval by an independent internal local ethical research board/committee (IRB-No. 258/15) and was registered on 15.11.2016 in/at the German Register for Clinical Trials (DRKS ID 00011267; https://www.drks.de/drks_web/navigate.do?navigationId=trial.HTML&TRIAL_ID=DRKS00011267).

Based on published criteria, confirmation of CRPS I/II diagnosis was achieved in the university's pain medicine center (anesthesiology, neurology) by a third independent investigator (3,4,6) and study eligibility in an interdisciplinary study board. Medication remained unchanged for the study subjects four weeks prior to study enrollment and during the entire three-month study period. All subjects provided informed consent and a study nurse performed saliva/serum sampling, score and stimulation parameter assessment at baseline and after three-months of unilateral L4-DRG_{STIM}. OXY saliva was assayed in addition after seven days of L4-DRG trial stimulation (Table 1 includes a summary of exclusion/inclusion criteria).

Data Collection and Characteristics of the Study Cohort at Baseline

The study cohort consisted of 24 subjects including 12 CRPS subjects eligible for L4-DRG (mean age: 70 ± 9.3 years; eight females and four males) and a suitably matched healthy control group (HC: mean age of 62 ± 16.9 years; nine females and three males). Impaired sleep quality was measured using the Pittsburgh Sleep Quality Index (PSQI) with a mean PSQI global score at baseline of 11 ± 8.5 along with depressive symptoms (Beck Depression Inventory BDI; baseline score: 17 ± 6.6) (43,44). The mean body mass index (BMI) for the study cohort was 29 ± 5.6 kg/m^2 (preobese) with three normal weighted subjects, five were classified preobese, obesity class I was present in one CRPS patient, obesity class II in two subjects, and obesity class III in one patient. At least one or more of the following metabolic disorders was present in all DRG subjects (hypertension, diabetes, cardiac ischemia). The average duration of

Table 1. Overview of Patient Selection (Exclusion/Inclusion) Criteria.

Inclusion criteria	Exclusion criteria
• Patient with confirmed chronic, intractable pain of the knee region (CPSP) not suitable for re-surgery, medical, and/or behavioral treatment and have been recommended by a multidisciplinary pain board for DRG spinal cord stimulation therapy • Patient is between 18 and 75 years of age at the time of enrollment • Patient must be willing to use DRG during his trial period (if applicable) • Unchanged medication four weeks prior to SCS-DRG implantation • No systemic inflammation (excluded by routine CRP/Procalcitonin screening)	• No informent consent • Concomitant neuropsychiatric comorbidity not adequate classified and/or requiring specific diagnosis/treatment • Pregnancy • Cardiac pacemakers • Malignancy • Previously performed invasive and ablative pain treatment

conventional multimodal pain therapy was 5.2 ± 0.3 years with all subjects having 3–14 knee joint interventions.

Protocol for Implantation Technique and Trial Period

At day 1 standard time (08.00–09.00 AM), baseline score data (Numeric Rating Scale for Pain NRS; PSQI, BDI, BMI) and saliva/serum samples were obtained. Afterward, all subjects received a CT-guided unilateral L4-DRG infiltration using a short-lasting anesthesia (4–6 hours) in order to confirm a sufficient coverage of the painful knee area and reproducible spine level for percutaneous lead placement with all subjects relapsing to their baseline pain levels within 24 hours (Fig. 1). Per patient one lead was permitted according to our study protocol. In all subjects, DRG L4 was determined as implant spine level. Adjustment of stimulation parameters was permitted per protocol within the study period.

At day 3, the DRG lead (AXIUM Neurostimulator System, Abbott Inc., Plano, TX, USA) was implanted under fluoroscopy guidance under an asleep protocol. The implantation was adopted to the previously described technique by Falowski et al. (45,46). Briefly, this technique enables the identification of the targeted DRG spine level using sensory and motor thresholds/responses quantified by somato-sensory evoked potentials and electromyogram (45,46).

The leads were externalized for a trial period lasting for seven days with a successful trial defined as at least 50% pain reduction compared to baseline. In a second procedure, the IPG (Proclaim, Abbott Inc., Plano, TX, USA) was placed in a subcutaneously prepared pocket and connected with the L4-DRG electrode. In case of failure, the leads were removed under local anesthesia.

Blood and Saliva Sampling

Saliva and blood samples were collected from CRPS patients at a standardized time (08.00–9.00 AM) at baseline and again after three-months L4-DRG under fasting condition. Saliva samples were obtained in addition after the trial period of seven days and were collected using prechilled salivettes (Sarstedt, Nuembrecht, Germany). Salivettes were immediately centrifuged at 4180 g for 2 min and aliquoted samples were stored at −80 °C until assayed. The peripheral venous blood was withdrawn at baseline and follow-up in the monovette serum gel tubes (Sarstedt, Nuembrecht, Germany). The blood was centrifuged at 3000 rpm for 10 min in a bench top centrifuge (Sigma, Osterode am Harz, Germany) after it

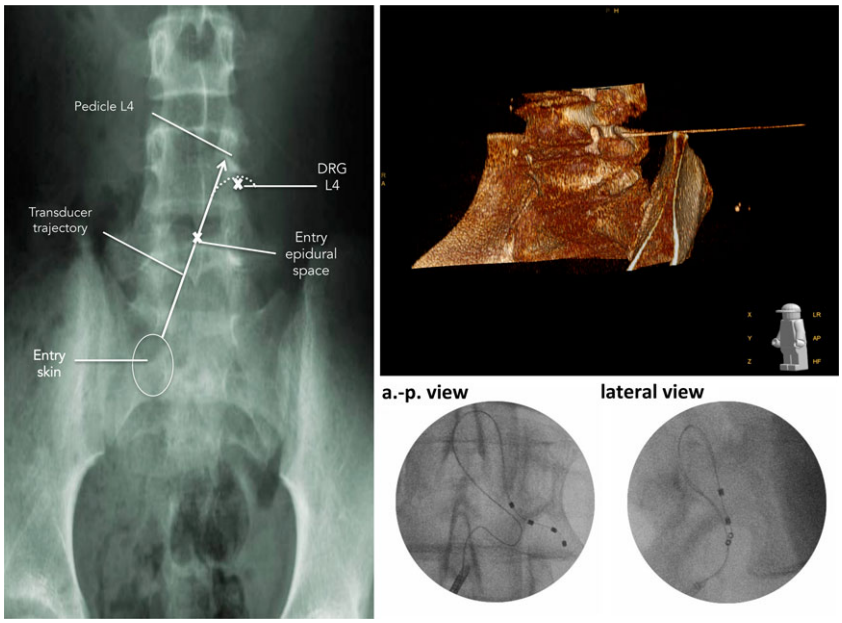

Figure 1. Left-sided a radiographic-based imaging with schematic drawing of the trajectory for percutaneous placement of the DRGSTIM. Right-sided upper row shows a 3-D reformatted CT scan showing the needle of the pre-implant infiltration at the level of L4. Lower row demonstrates postoperative lead placement control demonstrating appropriate L4 spine level implantation. [Color figure can be viewed at wileyonlinelibrary.com]

was allowed to clot for 15 min at room temperature. In a next step, the serum was aliquoted and preserved at −80 °C until analysis.

Saliva and blood samples also were obtained from a healthy control group (HC) matching the demographic characteristics of the treatment group. Healthy controls were recruited from the local population by means of online advertisement, public postings, and contacts to assisted living facilities. Subjects were free of any current physical or psychiatric illness as assessed by medical history. After completion of the study, participants received monetary compensation.

Enzyme-Linked Immunoassays of Neuroinflammation Markers in Serum and OXY in Saliva

Different cytokines: IL-1β, TNF-α, IL-6, IL-10, and HMGB1 were quantified in serum of the control and CRPS patients by high-sensitive enzyme-linked immunoassays (ELISA). Serum IL-1β, TNF-α, and IL-6 high sensitivity ELISA kits were employed to quantify the levels of these cytokines by following the manufacturer instructions (Catalog # HSLB00D, HSTA00E, and HS600B, respectively; R&D Systems, MN, USA). HMGB1 ELISA kit was supplied by IBL International (Catalog # ST51011, Hamburg, Germany) and was performed in high sensitive range 0.313–10 ng/mL. Serum IL-10 was quantified by BD OptEIA ELISA kit from BD Biosciences (Catalog # 550613, San Jose, CA, USA). The systemic levels of adipokines such as adiponectin, leptin, and ghrelin were quantified in the serum of the control and CRPS patients by enzyme-linked immunoassays. Human total adiponectin and leptin ELISA kits were obtained from R&D Systems (Catalog # DRP300 and DLP00, Minneapolis, MN, USA), while ghrelin serum levels were determined by ELISA kit obtained from eBioscience (Catalog # BMS2192, Bender MedSystems GmbH, Vienna, Austria). The serum levels of these adipokines were determined by following the manufacturers' instructions.

Salivary OXY concentrations were determined by using a 96 well commercial oxytocin ELISA kit (IBL, Hamburg, Germany). Measurements were performed in duplicate, and samples were treated following kit instructions. According to the manufacturer, the sensitivity limit of the assay is 11.7 pg/mL.

Statistical Analysis

The characters of the CRPS patients and healthy controls were presented as mean ± SEM depending upon the normality of the data distribution, which was assessed by Kolmogrov–Smirnov test or Shapiro–Wilk test. The levels of cytokines were analyzed among healthy, pre- and post L4-DRG_{STIM} group by one-way ANOVA or Kruskal–Wallis test followed by post hoc Dunns test for multiple comparisons. A p value <0.05 was considered as significant difference. The data were analyzed using GraphPad Prism 5.00 (San Diego, CA, USA).

RESULTS

Unilateral L4-DRG_{STIM} Effects on CRPS Pain Levels and Functional Impairment

After one-week L4-DRG_{STIM} trial phase 83.3% (10/12) were classified as responder (defined as ≥50% pain reduction compared to baseline) and received a permanent L4-DRG_{STIM} system. One trial was negative without substantial improvement with the lead being removed and in one patient lead placement was impaired due to spinal stenosis. CRPS associated pain declined significantly at three-month follow-up compared to baseline (mean NRS; pre-DRG_{STIM}: 74.90 ± 16.3 vs. one-week DRG_{STIM}: 42.50 ± 13.18 vs. three-months DRG_{STIM}: 46.65 ± 27.52; p = 0.003). A significantly disturbed sleep quality was present at baseline and after L4-DRG_{STIM} (mean PSQI; HC: 2.8 ± 2.2 vs. pre-DRG_{STIM}: 11 ± 8.5 vs. post-DRG_{STIM}: 9 ± 4.71; p = 0.0001). Compared to controls, a significantly disturbed mood state was found at baseline (mean BDI; HC: 4.6 ± 5.5 vs. pre-DRG_{STIM}: 17 ± 6.6; p = 0.0002), but not after three months adjunctive L4-DRG_{STIM} (mean BDI; post-DRG_{STIM}: 11 ± 8.53). The majority of the study cohort (9/12) exhibited a preobese (5/12) or obese (4/12) metabolic state compared to healthy controls with a mean BMI for HC: 24.2 ± 4.6 vs. pre-DRG_{STIM} 29 ± 5.6 vs. post-DRG_{STIM}: 27 ± 3.1 (Fig. 2).

Serum Concentration Changes of Pro-Inflammatory IL-1b, IL-6, TNF-α, HMGB-1, BDNF, Leptin

Compared to healthy controls, significantly increased serum values were detected pre- and post L4-DRG_{STIM} for HMGB-1 (HC: 1.2 ± 1.6 ng/mL vs. pre-DRG_{STIM}: 7.7 ± 10.14 ng/mL vs. post-DRG_{STIM} 4.3 ± 2.7 ng/mL; p = 0.0001), TNF-α (HC: 0.94 ± 0.3 pg/mL vs. pre-DRG_{STIM}: 1.72 ± 0.39 pg/mL vs. post-DRG_{STIM}: 1.71 ± 0.4 pg/mL; p = 0.0001), IL-6 (HC: 2.14 ± 2.47 pg/mL vs. pre-DRG_{STIM}: 5.61 ± 4.85 pg/mL vs. post-DRG_{STIM}: 5.54 ± 5.6 pg/mL; p = 0.0008), and leptin (HC: 23,666 ± 17,828.5 pg/mL vs. pre-DRG_{STIM}: 65,758.33 ± 69,321.69 pg/mL vs. post-DRG_{STIM}: 60,975 ± 58,537.67 pg/mL; p = 0.015), respectively. Serum concentration of IL-1b was significantly elevated pre-L4 DRG_{STIM} compared to healthy controls (HC: 0.09 ± 0.1 pg/mL vs. pre-DRG_{STIM}: 0.16 ± 0.1 pg/mL; p = 0.0178), but not post L4-DRG treatment (0.14 ± 0.1 pg/mL) (Figs. 3 and 4). BDNF serum levels were higher in CRPS subjects and remained unchanged after L4-DRG_{STIM} (HC: 31,424.18 ± 9326.80 pg/mL vs. pre-DRG_{STIM}: 39,425.40 ± 10,234.85 pg/mL vs. post-DRG_{STIM}: 38,699.21 ± 8054.56 pg/mL).

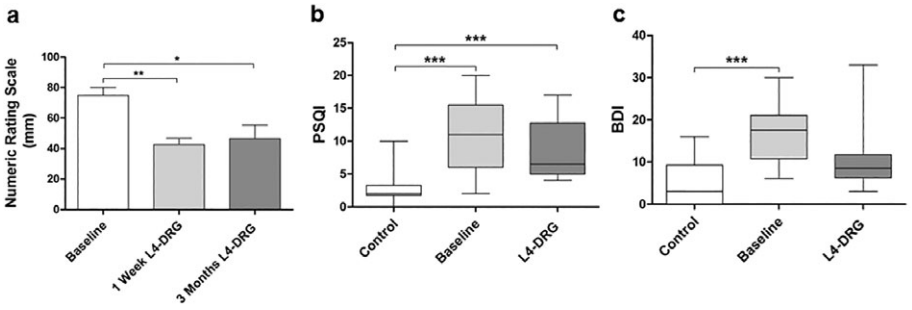

Figure 2. Numeric rating scale for pain (NRS), sleep quality (PSQI) and mood assessment (BDI): A comparison of baseline assessment and after 3 months selective L4-DRGSTIM (two right columns) of pain intensity compared to those of healthy controls (HC). Mean values with standard deviation and p-values. */**/*** indicates p-values < 0.05 (statistically significant).

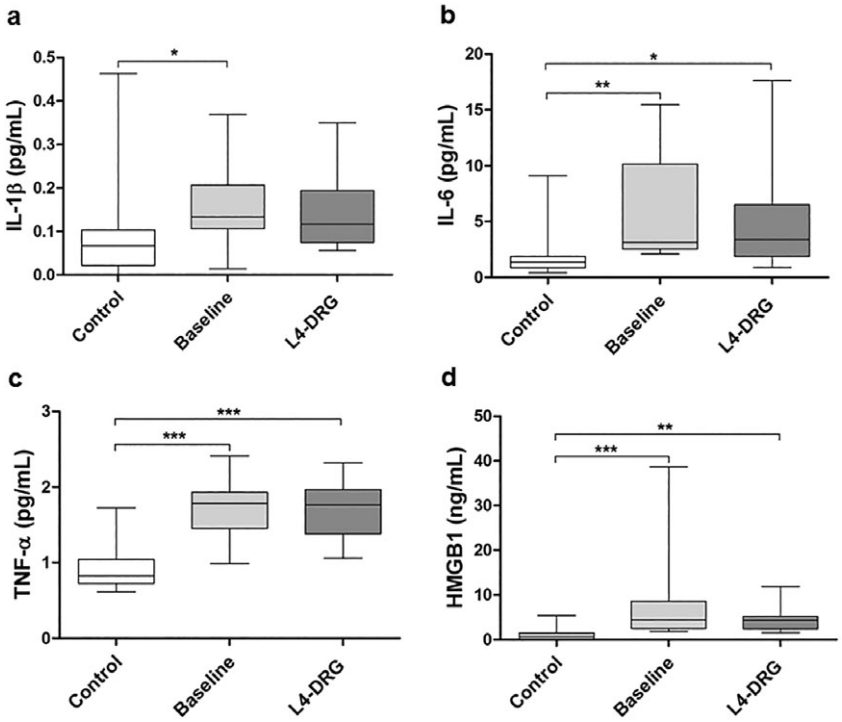

Figure 3. Interleukin-1β, interleukin 6, TNF-α and high-mobility group box 1 protein (HMGB1) serum analysis. A comparison of baseline assessment and after 3 months selective L4-DRGSTIM (two right columns) compared to those of healthy controls (HC). Mean values with standard deviation and *p*-values. */**/*** indicates *p*-values < 0.05 (statistically significant).

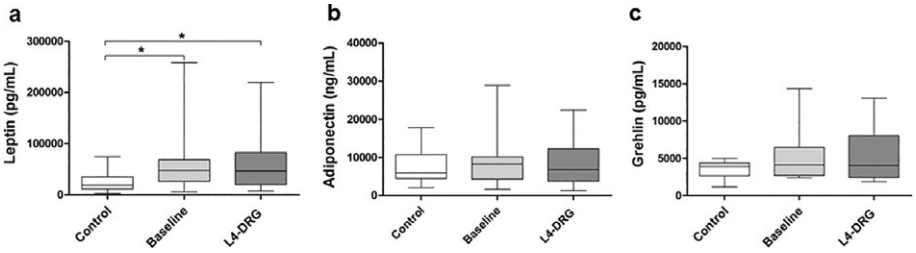

Figure 4. Leptin, adiponectin and ghrelin serum immunoassays. A comparison of baseline assessment and after 3 months selective L4-DRGSTIM (two right columns) compared to those of healthy controls (HC). Mean values with standard deviation and *p*-values. */**/*** indicates *p*-values < 0.05 (statistically significant).

Levels of Anti-Inflammatory Mediators in Saliva (OXY) and Serum (IL-10, Adiponectin, Ghrelin)

Levels of metabolic disorders associated anti-inflammatory mediators adiponectin (HC: 7391.67 ± 4144.78 pg/mL vs. pre-DRG_{STIM}: 8612.50 ± 7063.3 pg/mL vs. post-DRG_{STIM}: 8681.67 ± 6603.1 pg/mL) and ghrelin (HC: 3538.5 ± 1065.95 pg/mL vs. pre-DRG_{STIM}: 5307.5 ± 3715. 6 pg/mL vs. post-DRG_{STIM}: 5464.6 ± 3842. 9 pg/mL) remained statistically unchanged between controls, pre- and post L4-DRG_{STIM} CRPS subjects (Fig. 4). Elevated anti-neuroinflammatory IL-10 serum levels were found at baseline compared to healthy subjects and significantly decreased after three-month L4-DRG_{STIM} (HC: 13.78 ± 19.1 pg/mL vs. pre-DRG_{STIM}: 38.06 ± 29.71 pg/mL vs. post-DRG_{STIM}: 7.61 ± 8.12 pg/mL; $p = 0.0063$) (Fig. 5).

Saliva oxytocin concentration was slightly higher in CRPS patients compared to controls and increased after one-week L4-DRG_{STIM} trial and after three-month L4-DRG_{STIM}, although without statistical differences between all groups (HC: 30.45 ± 14.38 pg/mL vs. pre-DRG_{STIM}: 32.58 ± 13.0 pg/mL vs. post-DRG_{STIM} one week: 55.35 ± 75.01 pg/mL vs. post DRG_{STIM} three months: 59.82 ± 41.89 pg/mL; $p = 0.65$) (Fig. 5). C-reactive protein (CRP) values were low (average 0.34–0.48 mg/dL) measured according to the study protocol.

L4-DRG Stimulation Parameters

The stimulation parameters are given in Table 2: bipolar configuration, 20 Hz frequency, 200–300 μsec pulse width, stimulation intensities 300–1600 μA (Table 2).

Adverse Events

Within the study period no severe implantation and stimulation associated adverse events were recorded. Mild IPG pocket irritation occurred in one patient but resolved spontaneously. In one patient percutaneous placement was restricted due to coexisting spine fibrosis and in a second subject trial period was judged not positive (lead location misplacement or migration was excluded by postoperative imaging).

DISCUSSION

Summary of Score-Based Study Outcome and Comparison With Previous Clinical Trials

In summary, 83.3% (10/12) of our cohort perceived a ≥50% pain reduction after seven-day L4-DRG_{STIM} trial. After three months, there

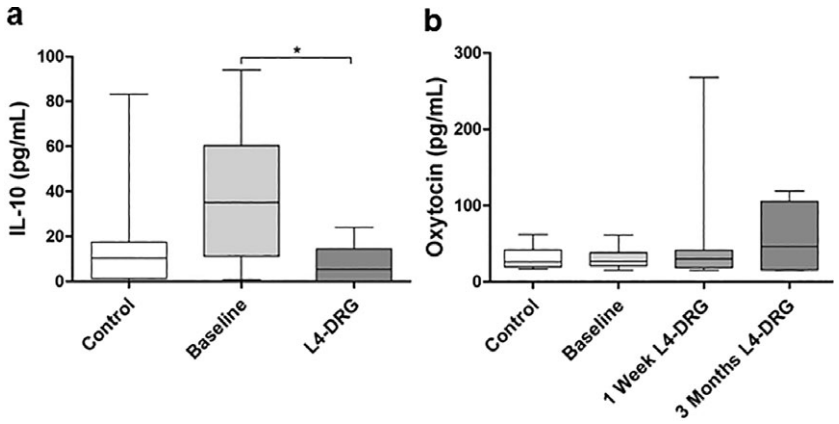

Figure 5. Serum levels of interleukin-10 and saliva concentrations of oxytocin at baseline, after 1 week L4-DRGSTIM trial and after 3 months. A comparison of baseline assessment and after 3 months selective L4-DRGSTIM (two right columns) compared to those of healthy controls (HC). For saliva oxytocin an additional measure was performed after 1 week trial stimulation. Mean values with standard deviation and *p*-values. */**/*** indicates *p*-values < 0.05 (statistically significant).

Table 2. Distribution of Patient Characteristics at Baseline: Age, Gender, CPSP Subtyp Diagnosis and Stimulation Parameters for Contact Configuration, Pulse Width, Frequency and Intensity.

Pat.	Gender (Age)	Spine level/side DRG	Activated contacts	Frequency (Hz)	Pulse width (μsec)	Amplitude (μA)	CPSP diagnosis	
1	W (75)	L4—left	1−; 3+	20	300	900–1200	CRPS	
2	M (71)	L4—right	1−; 2+	20	250	400–500	CRPS	
3	W (65)	L4—left	1+; 3−	20	400	500–600	CRPS	
4	W (72)	L4—right	2+; 4−	20	300	500–700	CRPS	
5	M (56)	L4—right	3+. 4−	20	200	1400–1600	CRPS	
6	W (50)	L4—left	1−; 2+	20	200	1200–1500	CRPS	
7	M(58)	L4—left	1−; 3+	20	300	600–750	CRPS	Negative trial
8	M (63)	L4—left	3+; 4−	20	200	400–1000	CRPS	
9	W (50)	L4—left	–	–	–	–	Causalgia	Implant failure
10	W (79)	L4—right	3+,4−	20	250	700–1400	CRPS	
11	W (53)	L4—right	2+, 3−	20	200	300–900	CRPS	
12	W (71)	L4—left	1−, 2+	20	200	350–550	CRPS	

was 61.3% pain reduction in the entire study cohort with 60% of the subjects having a sustained declined pain level of at least 50% (responders), whereas 40% of the DRG_{STIM} subjects relapsed (20–30% pain suppression compared to baseline) over the three months. Certainly, adjustment of pain medication and implantation of a second DRG_{STIM} lead (L3 or L5) may have improved responsiveness but was not permitted according to our study protocol.

Previously conducted CRPS trials using tonic SCS waveforms reported an overall success rate between 40 and 50%. Observational cohort studies and retrospective analysis found an increased response rate applying targeted DRG_{STIM} for neuropathic pain of the lower limbs ranging from 50 to 70%. The main diagnosis in the aforementioned trials included CRPS and other chronic pain condition (5).

The impact of both neuromodulation approaches/targets (spinal cord vs. DRG) was compared in a RCT-designed clinical trial (6). The ACCURATE trial observed a responder rate of 81% for DRG_{STIM} treated patient compared to 56% treated with conventional SCS (response threshold defined as at least 50% pain reduction) along with a global decline of pain levels of 84% for the DRG_{STIM} group. Contrary to our study, in the ACCURATE study, two DRG_{STIM} leads were permitted per patient in order to sufficiently achieve coverage of the affected pain area. Furthermore less postural disturbances and improved functional impairment (mood, quality of life) was observed for the DRG_{STIM} treated subjects (6). Of note, in assessing the different impact of tonic SCS and DRG_{STIM}, one should be aware of the fact that stimulation parameters, number of implanted leads (DRG 2 leads), composition of multilevel sensory influx via the DRG, and stimulation associated recruitment of neural fibers differ between SCS and DRG stimulation (dose–response-relationship). The success rate reported in our trial is in line with previously published DRG_{STIM} studies, but below the observed responsiveness of the ACCURATE trial (8). A significantly improved functional capacity (mood, quality of life) was observed in ACCURATE at three months for tonic SCS and DRG_{STIM} with superiority after 12 months in favor of DRG treated CRPS patients (6).

In our trial, sleep quality and mood was significantly impaired at baseline (pre-DRG treatment) compared to healthy controls as expected and improved post-DRG_{STIM} for mood, although statistically not significant.

Summary of Immunoassay-Based Study Outcome and Comparison With Previous Human and Preclinical Findings

A sufficient amount of preclinical studies addressed to the DRG indicate the pivotal role of inflammatory mediators (oxytocin, cytokines, chemokines) and their impact on DRG neural transmission in the genesis of neuropathic pain (17–37). Several

mechanisms (alteration of the membrane function [ion influx] or increased peptide expression leading to hyperexcitability in DRG and spinal cord neurons) may contribute to the maintenance or transition from acute to chronic neuropathic pain. Most of the uncontrolled observational human studies related to spinal neuromodulation and inflammation examined the effects of either tonic, or BurstDR SCS waveform on nociceptive back pain or neuropathic pain of the extremities as primary pain indication (41,42).

The antinoceptive potential of oxytocin has been well-documented (17–24). Several preclinical data have bridged the oxytocinergic descending hypothalamic-spinal circuit to antinociception and analgesia. In the brain, oxytocin is synthesized in neurons exclusively located within the hypothalamic nuclei (nucleus paraventricularis of the hypothalamus; PVN) and the supraoptic nucleus (SOP). Magnocellular neurons are distributed in the PVN and in the SOP, although in a higher number in the PVN. First, magnocellular neurons project to the posterior pituitary lobe (where oxytocin is released into the blood flow) and second, these neurons are connected with brain areas such as the amygdala, hippocampus, and cerebral cortex. A smaller population of parvocellular oxytocinergic neurons associated with the PVN, release oxytocin in the brainstem and spinal cord (dorsal column layers/DRG) but not in the systemic blood circulation (17–20).

Thus, through both pathways, oxytocin has been suspected to impact central and peripheral nociceptive transmission and inflammatory pain signaling. In particular, a suppression of A-delta/C-fibers activity in the spinal dorsal horn and the DRG was observed originating from the paraventricular nucleus of the hypothalamus (PVN). The exact mechanism remains not fully clarified, but oxytocin from the PVN may amplify GABA-nergic inhibition in the spinal cord (decreased neuronal activity at laminae I/II) and probably contributes to pain reduction via an opioid receptor-dependent mechanism (21–24). Saliva measurements of oxytocin have not been performed so far in human DRG stimulation or SCS trials. We observed a continuous increase more than three months in our cohort, although the changes were not statistically significant, it may in part reflect the observed DRG_{STIM} evoked pain reduction.

Contrary to a recently published BurstDR SCS—back pain—neuroinflammation study, we found an increased serum level of anti-inflammatory IL-10 at baseline, which declined significantly after three months unilateral L4-DRG_{STIM}. In line with previous tonic SCS and DRG-SCS human studies (uncontrolled), serum levels of pro-inflammatory mediators such as IL-1β, TNF-α, and IL-6 were significantly increased compared to healthy individuals within the entire study period. For instance, an elevated CSF level of BDNF was demonstrated in FBSS patients with predominant neuropathic leg pain under tonic SCS compared to healthy controls (41,42). BDNF, another inflammatory marker of neuropathic pain and depression, was higher in our chronic neuropathic pain cohort, but remained unchanged after L4-DRG_{STIM} (34,35).

Traumatic tissue/nerve destruction leads to local and systemic release of HMGB-1, a member of the alarmins protein family (DAMPs; damage associated molecular patterns), usually acting as intracellular transcriptional regulator. Extracellular HMGB-1 exposition activates macrophages/monocytes host response in the early phase of inflammation. To the best of our knowledge, this is the first study demonstrating increased serum levels of HMGB-1 in CRPS patients treated with L4-DRG stimulation (33,41,42).

Finally, as chronic pain and metabolic disorders (hypertension, diabetes, cardiac ischemia) co-occur in a considerable percentage of CRPS patients, we assessed markers associated with metabolic disorders and found significantly increased leptin serum levels at baseline and post L4-DRG_{STIM}, whereas markers of anti-metabolic disorder, adiponectin and ghrelin, did not vary between all groups. At least one or more metabolic disorder was diagnosed in each patient of our cohort and 75% of our study population was classified as preobese to obese class I–III. Only one BurstDR SCS study assessed metabolic markers in nonobese FBSS patients with predominant back pain and determined similar increased serum concentrations of leptin, a marker associated with the development of diabetes (insulin resistance), hypertension, heart failure, cardiac ischemia, and vascular architecture remodeling. Of note, IL-1β, TNF-α, and IL-6 represent promoters of those metabolic disorders, whereas IL-10 has been associated to counterbalance a pro-inflammatory metabolic state (41).

Given these facts, one should always have in mind that the mentioned cytokines have to be regarded in a multifunctional manner. For instance, IL-1β plays a critical role in neuropathic pain, metabolic disorders, heart failure, and depression. In chronic pain patients these symptoms/diseases are highly prevalent and should be considered in future neuroimmuno-modulation trials. Even in the absence of metabolic disorders, it may be worthy to assess their concentrations in order to identify patients at risk to develop such morbidities.

Limitations

A clear interpretation of the observed effects is hindered for several reasons. This study has several limitations including the uncontrolled design, the small-scale study cohort, lack of a sham control arm and short observation period. With respect to CRPS as a chronic multifactorial and complex pain condition, such expectation associated with a novel treatment (DRG stimulation) may also represent a confounder. The cytokine analysis performed in our study (measurement at two time points for serum and three time points for saliva) does not consider the dynamic nature of inflammation nor the circadian neurobiology, thus repetitive measurements should be considered.

An alternative, probably more sensitive methodology may be selective mRNA expression analysis of corresponding receptor domains with the capability to cover a broad range of inflammatory markers according to the underlying pain condition (nociceptive vs. neuropathic pain) (47).

CONCLUSIONS

However, these limitations may not negate the veracity or worthiness of the study; but certainly would have a different impact under a sham-controlled study design with long-term follow-up measures. The data was collected as a preliminary study to see which of various potential biomarkers would need to be collected to discern trends in neurostimulation therapy. Two different biofluids sources were investigated: blood, a more invasive approach in order to collect and handle; saliva, much more readily collected and potentially as effective in predicting inflammatory status and response to treatment.

Conclusively, adjunctive selective L4-DRG stimulation evoked pain relief and improved functional impairment in our CRPS cohort as reported in previous observational studies and the ACCURATE trial. The current study protocol failed to provide an evidence-derived conclusion about the predictive value and usefulness of saliva and serum assays due to its uncontrolled study design.

Chronic pain syndromes of different origin have been linked with an altered nociceptive neural signaling and a disrupted neuroimmune axis. Thus, future targeted clinical pain research should attempt to integrate molecular pattern assays relative to neuromodulation treatments (DRG, SCS). Beyond doubt, the inter- and intra-individual variability still remains an unmet issue in the field of applied neuromodulation and to this point, it is unclear to what extent it may impact neuromodulation treatment. Furthermore, it seems reasonable to combine such molecular measures with structural/functional neuroimaging, neurophysiological assessment (e.g., EEG, MEG, QST), and/or computational modeling. With this in mind, such first attempts may wave the direction toward the identification of quantitative measures and may become useful to better understand variations in pain phenotypes (enhanced patient selection), treatment outcome (responder vs. nonresponder), and stratification of stimulation protocols. Thus, the concept of a personalized and predictive neurostimulation therapy based on a comprehensive, preimplant mapping represents the next pivotal step in clinical neuromodulation research for pain.

Acknowledgments

We very much appreciate the efforts, the time, and the contributions of all investigators related to our work, especially those not listed as authors: Carolina Link, MD and Frigga Hönig (study nurse). Gratitude is expressed to all patients who participated in the study.

Authorship Statements

All authors were involved in the study design and participated in data collection and data analyses. All authors contributed to the development of this manuscript and provided their critique and their approval of the final draft for submission to Neuromodulation.

How to Cite this Article:

Gravius N., Chaudhry S.R., Muhammad S., Boström A., Gravius S., Randau T., Scheele D., Westhofen P., Kruppenbacher J., Stoffel-Wagner B., Maier C., Weidlich A., Yearwood T.L., Chakravarthy K.V., Kramer J.M., Hurlemann R., Kinfe T.M. 2018. Selective L4 Dorsal Root Ganglion Stimulation Evokes Pain Relief and Changes of Inflammatory Markers: Part I Profiling of Saliva and Serum Molecular Patterns.
Neuromodulation 2018; E-pub ahead of print. DOI:10.1111/ner.12866

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