Decreased Low Back Pain Intensity and Differential Gene Expression Following Calmare®: Results From a Double-Blinded Randomized Sham-Controlled Study

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Abstract: In this double-blinded, randomized controlled trial we evaluated the effects of Calmare®, a non-invasive neurocutaneous electrical pain intervention, on lower back pain intensity as measured by the "worst" pain score and on pain interference using the Brief Pain Inventory-Short Form, on measures of pain sensitivity assessed by quantitative sensory testing, and on mRNA expression of pain sensitivity genes. Thirty participants were randomized to receive up to 10 sessions of Calmare® treatment ($n = 15$) or a sham treatment ($n = 15$) using the same device at a non-therapeutic threshold. At 3 weeks after conclusion of treatment, compared with the sham group, the Calmare® group reported a significant decrease in the "worst" pain and interference scores. There were also significant differences in pain sensitivity and differential mRNA expression of 17 pain genes, suggesting that Calmare® can be effective in reducing pain intensity and interference in individuals with persistent low back pain by altering the mechanisms of enhanced pain sensitivity. Further study of long-term pain outcomes, particularly functional status, analgesic use and health care utilization, is warranted. © 2015 Wiley Periodicals, Inc.

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Persistent low back pain is one of the nation's most expensive medical conditions and a leading cause of disability. Of the $30 billion spent annually on direct care expenditures for low back pain (LBP), approximately 95% goes toward the treatment of individuals who develop persistent LBP (Soni, 2011). Even with conventional treatments that include psychobehavioral and pharmacological therapeutic options, over 80% of individuals who develop persistent LBP will continue to have pain for >12 months (Institute of Medicine, 2011). It is the most common site of pain in young and middle-aged adults and one of the most frequent reasons for sick leave and long-term time out of employment (Burgoyne, 2007). With a substantial increase in the prevalence of persistent LBP over the past decade, there has also been a rise in the use of epidural injections, surgery, and opioid medications, interventions that carry a risk of complications and untoward side effects (Freburger et al., 2009). The identification of safe and effective pain management strategies is a national priority and important goal for individuals with persistent LBP, a condition that can have a significant negative impact on quality of life and health status (Institute of Medicine, 2011).

Nurses play a critical role in the assessment of pain and provision of symptom management strategies. Although physical activity is a mainstay of persistent LBP treatment and important component of self-management, pain intensity is a major limiting factor for engaging in physical activity (Chou & Huffman, 2007). Building an evidence base on alternative non-pharmacological modalities, such as the Calmare® device tested in this study, can help guide the selection of therapeutic options for reducing pain intensity and improving function. Moreover, simultaneously

investigating the effect of the intervention on underlying pain mechanisms could provide foundational knowledge for advancing the delivery of personalized symptom management for individuals with persistent LBP.

Interrupting the Mechanisms of Persistent Pain

Several lines of evidence support the premise that persistent LBP develops as a consequence of enhanced pain sensitivity, an altered state of pain processing that augments pain and impairs descending pain inhibition. Physiological changes that lead to enhanced pain sensitivity include sensitization of nociceptors and neuronal circuits (Freburger et al., 2009; Giesecke et al., 2004) and modifications in the expression of genes that encode pain signaling molecules and their receptors, particularly genes associated with neurotrophins (Jacobsen, Eriksen, Pedersen, & Gjerstad, 2010; Nicol & Vasko, 2007; Pezet & McMahon, 2006), inflammatory mediators (Ren & Dubner, 2007) and catecholamines (Emery, Young, Berrocoso, Chen, & McNaughton, 2011; Gold & Gebhart, 2010). Further elucidation of the molecular events leading to reduced LBP could help to guide the integration of non-pharmacological strategies for symptom management.

Neurocutanous electrical stimulation may alter the release of pain-promoting molecules in the periphery and thereby modulate peripheral nociceptive function (Hulse, Donaldson, & Wynick, 2012). The Calmare® device was designed to interrupt the mechanisms of persistent pain by transforming, or scrambling, the pain signaling messages initiated by damaged/injured nerve fibers (Sabato, Marineo, & Gatti, 2005). Using biophysics-derived algorithms, the device transmits electrical waveforms that mimic endogenous action potentials that are recognized as non-pain information by damaged nerve fibers. The ability to modify action potentials of the affected nerve fibers into non-pain sensory information is unique. In particular, the Calmare® device generates nonlinear waveforms, as opposed to the linear waveforms of transcutaneous electrical nerve stimulation (TENS). In addition, the waveforms are wide and therefore able to stimulate the C-fibers as well as A-beta fibers and dynamically sequenced so that the nerve fibers cannot adapt.

The safety and efficacy of Calmare® was demonstrated in a trial that included 226 patients with idiopathic pain including LBP, trigeminal neuralgia and complex neuropathies (Sabato et al., 2005). Of these, 80% responded with $>$50% pain relief, 10% responded with pain relief from 25% to 49%, and 10% had no response. A subsequent trial included 52 patients with chronic neuropathic pain who were randomized to Calmare® or standard pharmacological treatment (Marineo, Iorno, Gandini, Moschini, & Smith, 2012). The enrollment mean pain intensity score on visual analog scale (VAS) was 8.1 and decreased at 1 month in the Calmare® group to .7 ($-$91%) and the standard therapy group to 5.8 ($-$28%). After 2 and 3 months, the Calmare® group mean VAS remained low at 1.4 and 2.0 respectively while there was minimal change in the standard therapy group. Similar findings on the effect of Calmare® on pain intensity have been reported for patients with chemotherapy-induced peripheral neuropathy and postherpetic pain (Coyne, Wan, Dodson, Swainey, & Smith, 2013; Ricci et al., 2011; Smith, Coyne, Parker, Dodson, & Ramakrishnan, 2010; Smith & Marineo, 2013).

Although the results of these trials have been impressive, they have all been open-label and thereby provided limited information regarding placebo analgesic effects, which can confound interpretation of the intervention's unique effectiveness. In addition, no investigators have examined the influence of Calmare® on pain sensitivity, a physiological mechanism involved in the refractory nature of LBP (Clauw et al., 1999; Jankowski & Koerber, 2010; O'Neill, Manniche, Graven-Nielsen, & Arendt-Nielsen, 2007). Although the placebo effect can have a significant impact on subjective pain scores, one would not expect to observe a change in pain sensitivity or mRNA expression of pain sensitivity genes with administration of a sham intervention (Gracely, 2005). Previous studies in animal models exposed to nerve injury have demonstrated decreased pain sensitivity in response to neurocutaneous electrical stimulation (Cavalcante Miranda de Assis et al., 2014; Yang, Yang, & Gao, 2010). However, the effect of electrical stimulation on peripheral mediators of pain sensitivity has not been previously examined.

Therefore, this study was designed as a double-blinded randomized sham-controlled trial in order to evaluate the effects of Calmare® on pain intensity and inteference, measures of pain sensitivity, and mRNA expression of pain sensitivity genes in individuals with persistent LBP. The primary aim was to compare the intensity of LBP and pain interference, measured by the Brief Pain Inventory-Short Form (BPI-SF), between participant groups randomized to Calmare® or sham over time. The secondary aims were to evaluate the effect of Calmare® on pain sensitivity in response to noxious stimuli and on mRNA expression of candidate genes involved in the transduction, maintenance, and modulation of pain responses.

Methods

Design and Setting

The study used a parallel-group, simple randomization scheme with 1:1 ratio and was conceptualized as a superiority trial to examine whether Calmare® provided more pain relief than sham at 3 weeks after treatment completion. The trial took place at a large, urban academic health care system in the mid-Atlantic region where an average of

10,657 patients with LBP is seen each year. The subjects of the study were recruited from March to June 2013.

Participants

Individuals between the ages of 18–50 years of age who were diagnosed with persistent nonspecific LBP were invited to participate through referrals from their primary health care provider. For the purpose of the study, persistent nonspecific LBP was defined as pain without a specific cause or need for surgical intervention, located anywhere in the region of the low back bound superiorly by T12 and inferiorly by the buttock crease, which had been present for $\geq$3 months duration for $>$4 days/week and was rated at an intensity level of $\geq$4 on the numerical pain scale (from 1 = *no pain* to 10 = *pain as bad as one can imagine*).

Eligible participants were: (i) between 18 and 50 years of age; (ii) diagnosed with persistent nonspecific LBP; and (iii) able to read and speak English. The age range was designed to provide a homogenous sample in terms of general health, work status, and contributing factors of persistent LBP.

Patients were excluded for the following conditions during the screening process: (i) chronic pain at another site or associated with a painful condition (e.g., fibromyalgia, rheumatoid arthritis) due to possible confounding factors on the analysis of mRNA expression of pain sensitivity genes; (ii) pregnant and/or breastfeeding due to unknown potential effects on the fetus and breast milk; (iii) latex allergies due to risk of skin hypersensitivity reactions with placement of the electrodes; (iv) skin conditions such as open sores that would prevent proper application of electrodes; (v) previous treatment with Calmare® or intolerance to transcutaneous electronic nerve stimulation. Due to potential interference of the electrical stimuli on physiological functioning of underlying organs and other implanted devices, the following exclusion criteria were also applied: (vi) severe arrhythmia or any form of equivalent heart disease; (vii) history of myocardial infarction or ischemic heart disease within the past 6 months; (viii) history of epilepsy, brain damage or use of anti-convulsants; (ix) prior celiac plexus block or other neurolytic pain control treatment within 4 weeks; (x) active withdrawal from drugs and/or alcohol; (xi) implanted drug delivery system (e.g., Medtronic Synchromed™); and (xii) pacemakers, automatic defibrillator, aneurysm clips, vena cava clips, or skull plates.

Among the 67 people screened for the study, 30 met the inclusion criteria and agreed to participate. Using a computerized random number table, the participants were assigned to either the Calmare® intervention group ($n = 15$) or sham control group ($n = 15$) by an intervention-designated research assistant (IDRA) who did not perform data collection or have access to study data. The IDRA kept a running list of the participant number and randomization group allocation in a locked cabinet until the study was complete. The remaining study team members and participants were blinded to the randomization scheme. There were no dropouts in either group. The progress of the participants through the study is shown in Figure 1.

Power analysis. The primary endpoint was the change in the "worst" pain score measured by the BPI-SF from baseline to 3 weeks post-intervention. Using an anticipated effect size (Cohen's *d*) of 1.58 determined from a previous study (Marineo et al., 2012), with a statistical power level of .8 and probability level of .5, the minimum total sample size for a two-tailed hypothesis is 16, with 8 participants per group. Thus, the planned sample size of 30 participants with 15 in each group was deemed appropriate.

Measures

Demographics. Age, gender, race/ethnicity, employment, socioeconomic status, educational attainment, and lifestyle behaviors (smoking, exercise) were collected at enrollment.

Pain intensity and interference. The BPI-SF consists of 15 questions that measure pain location, intensity, pain treatment, treatment effectiveness, and functional interference from pain (Keller et al., 2004). The BPI-SF assesses pain intensity at its "worst," "least," and "average," over the past week, as well as pain "now," using a numerical rating scale from 0 = *no pain* to 10 = *worst possible pain imaginable*. A lower score on any of these items reflects less pain. Pain interference is measured on the BPI-SF by asking how much pain has interfered with seven daily activities (general activity, walking, work, mood, enjoyment of life, relations with others, and sleep). Pain interference for each daily activity is rated on a 0–10 scale and scored as the mean of the seven items; a lower score represents less interference. The BPI-SF has been validated in individuals with low back pain, demonstrates sufficient reliability (coefficient alpha $>$.70) and sensitivity to change over time (Keller et al., 2004).

Based on previous studies, our hypothesis was that at the 3-week follow-up visit, the group randomized to Calmare® would have significantly lower "worst" pain and interference scores compared to the sham group. The "worst" pain and interference scores of the BPI were used as the primary endpoint, as suggested by the IMMPACT recommendations for assessing pain in clinical trials (Dworkin et al., 2005). The "worst" pain score was selected a priori, as it has excellent test–retest reliability ($\alpha = .93$; Cleeland, 2009) and reflects temporal pain variability, a dimension of the pain experience that is heavily influenced by pain sensitivity.

Pain sensitivity. Pain sensitivity was measured using a standardized quantitative sensory testing (QST) protocol in which study participants were asked to rate their level of pain in response to three types of noxious stimuli

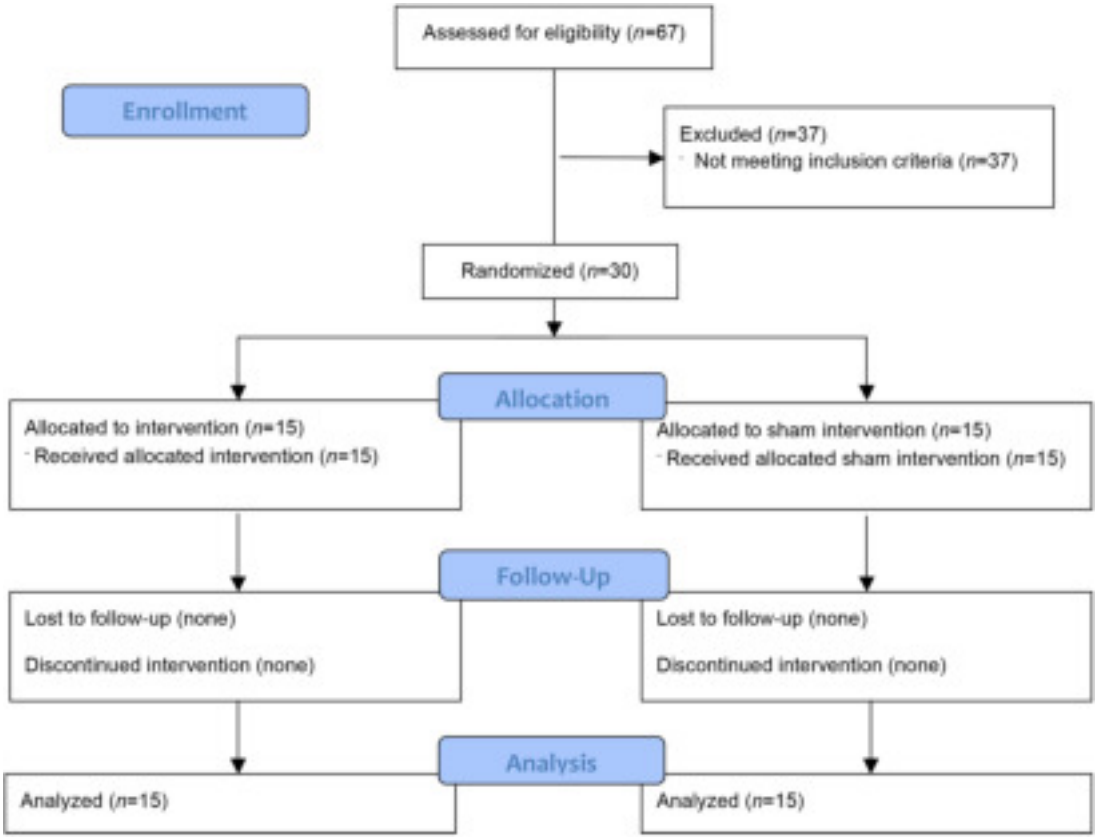

FIGURE 1. CONSORT participant flow diagram.

(Rolke et al., 2006). Test–retest and inter-rater reliability of the QST protocol has been reported in several clinical trials, with each individual test achieving a CI of .87–.94 (Chesterton, Sim, Wright, & Foster, 2007; Moss, Sluka, & Rice, 2007). Normalized age and gender-specific data acquired by our laboratory were used to assess the presence of pain sensitivity.

First, heat pain threshold was assessed on the participant's forearm and painful area of the back, by applying a thermode (Medoc Pathway System™; Durham, NC) that increased in temperature at a rate of .5°C/second. Threshold was defined as the temperature at which pain was first reported. A lower heat pain threshold is consistent with enhanced pain sensitivity. Next, rating of pain in response to a single thermal stimulus of 48°C applied to the painful area of the back was evaluated. A higher rating represents a higher level of pain intensity and enhanced pain sensitivity. Finally, pressure pain thresholds in response to a handheld pressure algometer were assessed on the painful area of the back. Pressure threshold was recorded as the algometer loading (kPa) at which pain was first detected. Similar to the heat pain threshold, the amount of stimulus (pressure) required to produce the first onset of pain was recorded; a lower value is consistent with enhanced pain sensitivity.

Candidate gene expression. At each data collection visit, whole blood was collected by venipuncture into one 5 ml EDTA vacutainer and one 10 ml cell preparation tube with sodium citrate, labeled with a unique study identification label, and transported directly to the laboratory for processing. RNA isolation was performed using the LeukoLOCK™ total RNA isolation system (Applied Biosystems, Carlsbad, CA) according to the manufacturer's protocol and was reverse transcribed using SuperScript VILO cDNA synthesis kit (Invitrogen, Valencia, CA). The mRNA expression of 84 genes involved in the transduction, maintenance, and modulation of pain responses was determined (Neuropathic & Inflammatory RT^2 Profiler PCR Array; Sabio Sciences, Valencia, CA) using qPCR performed on the BioRad CFX96®. After an initial incubation step, 35 cycles (95°C for 15 seconds and 1 minute at 60°C) of PCR were performed. Expression levels were quantified using the $^{\Delta\Delta}C_t$ method, which normalizes data of the genes of interest to β-actin (controls are included in array). The BioRadCFX software was used to determine optimal baseline and threshold settings of the assay C_t values.

Procedures

Prior to the intervention, data collection took place in a private research suite, where the participant was asked to complete study questionnaires, have blood drawn, and undergo sensory testing by a designated research assistant who was blinded to group assignment. All study questionnaires were self-administered. The laboratory personnel involved in processing of genetic assays were blinded to the randomization of the study participants throughout the study and at the time their genetic measures were quantified.

Upon completion of baseline study measures, the participant received a clinical pain examination, and the assigned intervention was initiated by the intervention-designated research assistant (IDRA), who was trained in administering Calmare®. The IDRA followed a script to explain the study intervention and provide instructions to each participant. During the clinical exam, the IDRA asked the participant to identify the most painful area of the back and to point to the confines of the area of pain.

Electrode placement and device settings. The number of electrodes used and placement of the electrodes were dependent on the study group assignment. For participants randomized to Calmare®, two pairs of electrodes were placed in the same dermatome associated with the most painful area, approximately 25 mm outside of the outer confines of the most painful area. During each 30 minutes session, the settings were increased on the device by 10 U every 20 seconds, to a maximum of 70 U. The sham group had one set of electrodes placed in the dermatome above the most painful area. The settings were increased from 0 to 10 U and remained at 10 U for the duration of each session.

Ten 30-minute sessions were administered over 10 working days or until the participant reported no pain, in which case the treatment ended and they were scheduled for a 1 week follow-up visit. A daily log and schematic diagram of electrode placement was maintained to ensure consistent delivery of the intervention. Participants were scheduled for follow-up data collection at 1 and 3 weeks after their final study treatment sessions. The same protocol for administering study questionnaires, performing blood draws and QST was followed for each follow-up data collection visit.

Ethical Considerations

The study was approved by the Institutional Review Board at a university before the study began, and the participants gave written informed consent in a face-to-face interview. Participant confidentiality was assured throughout the study period. Each participant was assessed for adverse events during and after each session and at each study visit. All participants were told that they had the right to withdraw from the study at any time.

Statistical Analysis

Descriptive statistics were calculated for the demographic characteristics of the participants and to assess the homogeneity of both groups, using Chi-square for all categorical variables and Student's *t*-test for continuous variables. To analyze the effectiveness of the intervention, a series of repeated-measures analyses of variance (ANOVA) were performed, with the "worst" pain and interference scores as the dependent variables, and time (baseline, 1-, and 3-week follow-up visit), group (Calmare® or sham), and group by time interaction terms as the independent variables. The repeated measures model accounts for the correlations that might arise from observations of the same individuals over time. Data were analyzed using SPSS statistical package (version 18.0 Chicago, IL), and the level of significance was set at $p < .05$.

Secondary endpoints included heat and pressure pain thresholds and mRNA expression levels of the 84 candidate genes. For all housekeeping genes included in the assay (ACTB, B2M, GAPDH, HPRT1, and RPLP0), the mean and standard deviation of Cq values across all samples was calculated. ACTB exhibited the lowest variance and highest abundance and was therefore used as the housekeeping gene for normalization. For the 84 candidate genes, the ΔC_t values for each timepoint t were calculated as $\Delta C_t = Cq_{GOI,t} - Cq_{ATCB,t}$. Thereafter, for each subject, the relative-fold change in expression at 3 weeks post-intervention with respect to baseline was calculated as $2^{-\Delta\Delta Ct}$, where $-\Delta\Delta C_t = \Delta C_t - \Delta C_1$.

For each of the 84 genes, a random coefficient model including treatment group (Calmare® vs. sham) and time (baseline vs. 3 weeks post-intervention) as fixed effects was fit. A likelihood ratio test was used to test the null hypothesis that the treatment group effects were zero (Hedeker & Gibbons, 2006). A $p < .01$ threshold was used to determine whether Calmare® and sham expression profiles significantly differed.

Results

The demographic characteristics of the participants in each group did not differ significantly, as shown in Table 1. Most participants were working part- or full-time, had a college-level education, and reported LBP duration between 6 and 12 months. In both groups, 80% of participants reported using non-steroidal anti-inflammatory drugs (NSAIDs) intermittently for pain (<4 times per week), and 20% reported using NSAIDs more regularly (>4 times per week).

At the 1- and 3-week follow-up visits, there was a statistically significant difference in the "worst" pain score between the Calmare® and sham groups (Table 2). Similarly, pain interference was significantly different between groups at the 3-week follow-up visit, with significantly lower pain interference in the Calmare® group. The "worst" pain and interference scores of the BPI showed a significant decrease in the Calmare® group from baseline to the 3-week follow-up visit, whereas the scores of the sham

treatment group did not change over time. In the Calmare® group, seven (47%) participants had a >50% reduction in the "worst" pain score from baseline to the 3-week follow-up visit, five (33%) participants had a 30–49% reduction, and three (20%) had a 20–29% reduction.

Measures of pain sensitivity (heat pain threshold, single stimulus rating, and pressure pain threshold) were significantly different between groups at the 3-week follow-up visit (Table 3). The higher-level thresholds to heat pain and pressure pain in the Calmare® group at 3 weeks reflected the higher-stimulus intensity required to cause a perception of pain. Consistent with less pain sensitivity, they also rated their perception of pain with the single heat stimulus aslower. Although the Calmare® group showed less pain sensitivity compared to the sham group at the 3-week follow-up visit, the within-group changes in pain sensitivity measures in the Calmare® group from baseline to 3 weeks did not reach a level of statistical significance.

Differential expression of 10 candidate genes between the Calmare® and sham groups was observed between baseline and 3 weeks post-intervention, while baseline mRNA levels of the 84 candidate genes did not differ. Using a *p*-value threshold of <.01, the fold regulation of the following genes was significantly different between the Calmare® and sham group: *BDKRB1*, *CACNA1B*, *CHRNA4*, *GDNF*, *GRM1*, *NGF*, *NTRK1*, *OPRD1*, *PENK*, and *PLA2G1B*. Table 4 shows the differential fold regulation in the Calmare® group at 3 weeks post-intervention.

In exploration of the success of blinding of participants to treatment assignment, both Calmare® and sham groups reported a significant reduction in pain in within-group analyses during the 10-day intervention period, as measured by a numerical rating scale administered by the IDTA before each treatment session, consistent with short-term placebo analgesic effects of the sham control. We also asked whether participants believed they had received Calmare® therapy (and not the sham treatment) after the three-week follow-up appointment. Participants were asked to respond using one of three categories: "definitely not," "unsure," or "definitely." In the sham treatment group, 66%

Table 1. Baseline Demographic Comparison of the Calmare and Sham Groups

	Calmare® ($n=15$)		Sham ($n=15$)		
Characteristic	Mean	Range or *SD*	*Mean*	Range or *SD*	*t*
Age	42.5	28–50	45.0	31–50	.80
Sleep (hours/night)	7.0	.9	6.2	2.0	1.41
	n	%	*n*	%	χ^2
Sex					
Female	8	53	11	73	.57
Male	7	47	4	27	
Race/Ethnicity					1.48
Hispanic	0	0	3	20	
Non-Hispanic	15	100	12	80	
African American	1	6	4	27	
Caucasian	13	88	10	67	
Other	1	6	1	6	
Marital status Married	5	33	6	40	.20
Single	5	33	5	33	
Divorced/widowed	5	33	4	27	
Income					.57
<$60,000	4	27	7	47	
>$60,000	11	73	8	53	
Employment					.50
Full-time/part-time	14	94	15	100	
Unemployed	1	6	0	0	
Education (highest level)					.54
High school/technical	2	13	0	0	
College	13	87	15	100	
Duration of low back pain	9	60	9	60	.14
6 months-1 year	6	40	6	49	
>1 year					
Current smoker					.29
Yes	1	6	3	20	
No	14	94	12	80	

Table 2. Worst Pain and Interference Scores in Calmare® and Sham Treatment Groups Over Time

		Calmare® ($n = 15$)		Sham ($n = 15$)			
Assessment	Time	Mean	*SD*	Mean	*SD*	*F*	$p_{group \times time}$
"Worst" pain	Baseline	5.40	1.52	4.98	2.11	.56	.45
	1 week follow-up	4.34	1.22	5.76	1.34	9.39	.01
	3 weeks follow-up	3.23[a]	1.27	5.81	1.75	23.42	<.01
Pain interference	Baseline	3.19	1.64	3.08	1.52	.03	.86
	1 week follow-up	2.75	1.87	3.04	1.91	.19	.66
	3 weeks follow-up	1.92[a]	1.36	2.89	1.19	4.18	.05

SD, standard deviation.
[a]$p < .05$ for within-group comparisons to the baseline score.

(10/15) responded that they had definitely received Calmare® therapy, 20% (3/15) were unsure, and 13% (2/15) responded definitely not. Similar response rates were obtained from participants in the Calmare® group.

Discussion

The primary aim of this study was to compare the intensity and pain interference of LBP over time, measured by the BPI-SF, between participant groups randomized to Calmare® or sham. The Calmare® group reported significantly lower pain intensity, as measured by the "worst" pain score, at the 1- and 3-week follow-up visits, and lower pain interference at the 3-week follow-up. The finding of decreased pain intensity after Calmare® is consistent with previous investigations in participants with other types of chronic pain, although different pain indicators, such as pain "now" or "average," were used in those studies (Coyne et al., 2013; Ricci et al., 2011; Smith et al., 2010; Smith & Marineo, 2013).

The presence of short-term placebo analgesic effects in response to the sham control supports the use of the sham protocol. Placebo analgesic responses are modulated through expectations regarding pain treatment and are regulated through responses to noxious stimuli in the spinal cord and brain as well as activation of descending pain inhibitory pathways (Benedetti, 2009). While it may be assumed that these effects also contributed to the change in pain scores of participants who received the Calmare® intervention, the effects of the neurocutaneous electrical stimulation appeared more gradually. In particular, although the Calmare® group did not have a statistically significant decrease in the "worst" pain score from baseline to 1 week post-intervention, the reduction was significant at 3 weeks post-intervention. Given that the "worst" pain score reflects temporal pain variability, or the "memory" of pain, these findings suggest the Calmare® intervention may influence peripheral and central pain processing.

Other factors that may have influenced this finding were the duration of the intervention and the timing of data collection. We used a duration of 30 minutes, but Calmare® may be used up to 45 minutes per session. In studies involving other forms of neurocutanous electrical stimulation in animal models, variation in the effects of electrical stimulation were found that were time-dependent and related to the electrical frequency of the stimulation (Cavalcante Miranda de Assis et al., 2014; Yang et al., 2010). Thus, identifying an optimal intervention and follow-up protocol may play an important role in the evaluation of the Calmare® intervention.

The secondary aim of this study was to evaluate the effect of Calmare® on pain sensitivity and mRNA expression of candidate genes involved in the transduction,

Table 3. Pain Sensitivity in Calmare® and Sham Treatment Groups Over Time

		Calmare® ($n = 15$)		Sham ($n = 15$)			
QST Measure	Time	Mean	*SEM*	Mean	*SEM*	*F*	$P_{group \times time}$
Heat pain threshold (C) (range 32–50)	Baseline	42.86	.72	42.23	.78	.35	.55
	1 week follow-up	43.45	.74	42.39	.78	.97	.33
	3 weeks follow-up	44.34	.76	41.41	.82	6.87	.01
Single stimulus rating (48ºC) (range 0–100)	Baseline	47.71	1.25	47.80	1.36	.00	.96
	1 week follow-up	46.65	1.26	48.83	1.36	1.38	.24
	3 weeks follow-up	46.00	1.32	50.22	1.45	4.63	.04
Pressure pain threshold (kPa) (range 100–600)	Baseline	378.52	38.78	390.68	41.89	.05	.83
	1 week follow-up	451.52	36.18	406.48	38.69	.72	.40
	3 weeks follow-up	479.16	40.34	354.25	43.91	4.39	.05

QST, quantitative sensory testing; SEM, standard error of the mean.

Table 4. Differential Gene Expression in the Calmare® Group at 3 Weeks Post-Treatment

Gene	Fold Regulation	p^a
BDKRB1	−2.468	.0069
CACNA1B	−1.518	.0091
CHRNA4	−1.924	.0053
GDNF	−2.141	.0036
GRM1	−1.715	.0033
NGF	−2.599	.0040
NTRK1	−1.980	.0035
OPRD1	−1.812	.0049
PENK	−1.850	.0042
PLA2G1B	−1.816	.0020

[a]Difference from sham treatment group.

maintenance, and modulation of pain responses. At 3 weeks post-intervention, the Calmare® group had significantly lower pain sensitivity scores compared to the sham group, yet the differences from baseline to 3 weeks within the Calmare® group did not reach a level of statistical significance, most likely due to the small sample size. In addition, the Calmare® group had significant downregulation of 17 pain genes at the 3 weeks follow-up visit, many of which code for proteins and receptors that mediate inflammation and nociceptive signaling. Increased peripheral expression of nerve growth factor (NGF) and glial-derived neurotrophic factor (GDNF), can potentiate primary afferent nerves and lead to peripheral sensitization (Malin et al., 2006). Expression levels of both neurotrophin genes were significantly lower in the Calmare® group compared to sham 3 weeks following the intervention.

In contrast, animal models exposed to nerve injury and then given electrical stimulation demonstrated an immediate reduction in pain sensitivity along with downregulation of pain-promoting molecules in the spinal cord and brain (Fang, Liang, Du, & Fang, 2013; Yang et al., 2010). However, nerve injury using animal models may not replicate the mechanisms of pain sensitivity that contribute to persistent LBP in humans, specifically changes in the rate of healing (Cavalcante Miranda De Assis et al., 2014). In addition, the differences in electrical frequency and dosage of the intervention may influence the physiological response at the peripheral and central levels (Cavalcante Miranda de Assis et al., Yang et al.).

Although we cannot infer that the modification in expression levels of these genes was responsible for the reduction in pain intensity, interference, and pain sensitivity, the findings do present an intriguing approach for exploring the systemic gene expression signature of a selected pain phenotype or, as in this case, response to treatment. Recently, gene expression profiles of peripheral blood leukocytes (PBLs) were evaluated in patients with symptomatic knee osteoarthritis (Attur et al., 2011). In that study, patients with knee osteoarthritis who had increased expression of interleukin (IL)-1β (>2-fold compared to controls) had higher pain scores, decreased function, and higher risk of radiographic progression of osteoarthritis. This approach has not been used to examine pain phenotypes of LBP or response to interventions. However, in a recent study of gene expression across different tissue types, including blood, skin, dorsal root ganglion, and slices of brain tissue, a consistent level of methylation or expression changes was found, which may make using gene expression profiles of PBLs possible in the future (Bell et al., 2014). Identifying the specific pain sensitivity genes that are most relevant to persistent LBP and those that contribute to pain relief will be a key factor in translating this approach into practice.

Taken together, the results of the study demonstrate a significant reduction in pain intensity, pain interference, and pain sensitivity at 3 weeks following the Calmare® intervention in comparison with the sham group. As nurses are heavily invested in the delivery of effective symptom management interventions, it is important to identify nonpharmacological strategies for reducing pain and improving function for individuals with persistent LBP. The present study demonstrates that Calmare® may significantly reduce pain intensity while receiving the intervention and lessen variability of LBP intensity and interference with activities over time. Because physical activity is important to prevent recurrent LBP and improve functional status, further investigation of Calmare® as part of an intervention for persistent LBP that includes physical activity and long-term functional outcomes will help to inform clinical practice.

Study Limitations

Several limitations of this study are worth noting, in addition to the small sample size. Although referrals to the study were made by clinicians who had previously diagnosed the participants with nonspecific low back pain, we did not review medical records or diagnostic imaging for confirmation. However, during the exam, particular attention was paid to detecting any red flags or signs of serious underlying pathology, and none were noted in the participant sample. In addition, the sham procedures of the protocol had not been previously evaluated. However, we did assess whether participants believed they had received Calmare® therapy (and not the sham treatment) after the three-week follow-up appointment, and similar responses were obtained from both groups. We used a candidate gene approach to assess differential gene expression. The candidate gene approach has inherent limitations of potentially not accounting for other genes relevant to pain or interventions, and changes in gene expression do not necessarily result in modifications at the protein level.

Conclusions

The results of this study suggest that Calmare® provides significantly more low back pain relief than sham at 3 weeks post-intervention and can reduce pain intensity, interference,

and pain sensitivity in individuals with persistent low back pain. The differential expression of pain genes between the intervention and sham group suggests that Calmare® may exert an effect by downregulating pain receptors and proteins involved in maintaining persistent pain. Further study is warranted of long-term outcomes after Calmare®, including functional status, analgesic use and health care utilization.

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