1. Clinical study report JAUNS 2015-121 (2015) The effect of HOTSHOT on muscle cramps. Statistical analysis of 120 subjects showed a significant reduction in muscle cramps when used compared to a control group. The study was conducted by Penn State University and presented at the annual meeting of the American Academy of Neurology (2015, Washington DC) (3).


**FIELD STUDY: CRAMP PREVALENCE & RETURN TO PLAY**

Of the 31 cramp-prone athletes, muscle cramps dropped over 50% during and after workouts when HOTSHOT was used 15–30 minutes before the workout. Athletes who did cramp returned to play faster than those who used a vehicle control. Statistical analysis showed a significant difference in cramp prevalence between the treatment and control groups. The effect lasted for 6–8 hours. HOTSHOT has been tested up to 2 hours.

**EXERCISE-ASSOCIATED MUSCLE CRAMPS & SORENESS**

Effect was shown in a double-blind, vehicle-controlled, crossover study of 20 subjects which maximally contracted one calf muscle until cramping occurred (4). This research was independently conducted by Penn State University and presented at the conference of Experimental Biology (2016, San Diego CA) (5).

**STUDIES SHOW SIGNIFICANT EFFECT**

**ELECTRICALLY INDUCED MUSCLE CRAMPS**

Effect was shown in multiple randomized, blinded, vehicle-controlled studies in which subjects consumed various formulations of TRP activators in a small beverage (1,2). This research was presented at the annual meeting of the American Academy of Neurology (2015, Washington DC) (3).

**CRAMP PREVALENCE DURING WORKOUT**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage</th>
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<tr>
<td>Muscle</td>
<td>43%</td>
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<td>Vehcile Control</td>
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**CRAMP PREVALENCE POST-WORKOUT**

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<tr>
<td>Vehicle Control</td>
<td>12%</td>
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**PAUSE OVER >5 MINUTES BEFORE CONTINUING**

<table>
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<tbody>
<tr>
<td>HOTSHOT</td>
<td>52%</td>
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<tr>
<td>Vehicle Control</td>
<td>25%</td>
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</table>
Chemical Neuro Stimulation of TRPV1 and TRPA1 Sensory Neurons Decreases Muscle Cramps in Humans

Glenn F. Short III, Laura B. Rosen, Robin Sutherland, Jian Liu, Jennifer M. Cermak, Gary Maier and Thomas Wessell
Flex Pharma, Inc. Boston, MA 02199

Summary

Chemical Neuro Stimulation is the treatment of neurological disorders by using small molecules applied topically to sensory neurons to alter the behavior of distinct neural circuits within the central nervous system. We have devised one such approach wherein the activation of TRPV1 and TRPA1 ion channels in the upper alveolar canal decreases muscle cramp frequency and severity. Based upon recent evidence that α-motor neuron hyperexcitability is the underlying cause of cramps and spasticity [1,2], we hypothesized that TRP activation could provide sufficient excitatory sensory input via the voluntary tract to modulate sensory processing pathways that increase muscular force and dampen motor neuron hyperexcitability. We have generated data that suggests that an oral solution containing a mixture of naturally derived TRP activators (TSP-Stim) or FLX-787, a synthesized single molecule TRPV1/TRPA1 co-activator, stimulate TRP channels in the mouth, oropharynx and esophagus in a local, topical fashion to inhibit muscle cramping. Efficacy studies using an electrically-induced cramp (ESC) model demonstrated that both TSP-Stim and FLX-787 significantly reduced cramp intensity by as much as 3-fold relative to control [p<0.01]. Moreover, the pharmacokinetic profile of FLX-787 could not account for its efficacy, as no systemic exposure of the parent form of FLX-787 in human plasma was observed. In both animals and humans, FLX-787 was found to undergo rapid phase 2 metabolism resulting in extensive catabolism 3 hours after ingestion, predominantly to glucuronide and sulfate metabolites. Even at doses up to 50 mg/kg/day in rats, the conjugates of FLX-787 accounted for <10% of circulating drug. To understand if topical exposure to nervous membranes in the mouth, oropharynx and esophagus mediated the TSP-Stim and FLX-787 effect (given the lack of systemic exposure to FLX-787), the TSP-Stim mixture was encapsulated in gelatin capsules. Ingestion of the encapsulated mixture afforded no ESC efficacy relative to vehicle control. These results suggest that the observed effect on electrically-induced muscle cramps does not depend on the systemic bioavailability of TRP activators but rather on topical exposure of sensory neurons and consequent neuronal signaling. That efficacious sensory signals have also been observed in proof of concept (POC) neomuscular leg cramp (NLC) trials with FLX-787. Chemical Neuro Stimulation may be a general approach to develop novel treatments for cramps, spasms and spasticity in clinical populations. Based upon these findings, we have initiated studies with FLX-787 in MS and ALS.

Methods

Muscle cramps were induced in the flexor hallucis brevis (FHB) muscle by electrical stimulation and assessed by blinded testers in quiescent sensory examiners of the ankle. (Figure 2). The subject’s ankle which was uninvolved was electrically stimulated through surface electrodes 5 mm above the experimentally induced cramp. The terminal of the cramp nerve was exposed (FHB) with release of characteristic electrical activity (Figure 2) Muscle cramps were quantified by blinded testers to as any first indication of electrical activity coincident with treatment administration to serve as a subject specific baseline control. After consumption of the FLX products or vehicle control, the resulting signals were quantified (time to onset, duration and intensity) and the time at which the subject described treatment or vehicle control to be the time post-צת.

Results

Figure 4. Topical exposure to oropharynx and esophagus is required for cramp inhibition

Figure 5. FLX-787 inhibition of electrically-induced cramps in healthy subjects by dosing-response

NLC Exploratory POC Studies

- **Nocturnal leg cramps (NLC)**
  - 50% of those over the age of 50 suffer from NLC with increasing prevalence and frequency with age. Over 4 million in the US over age 65 suffer daily.
  - Lack of clinical evidence that common “remedies” such as electrolyte replacement, bananas and hydration afford relief.
  - Quinine, prescribed in the United Kingdom for NLC, is associated with thromboocytopenia, hypersensitivity reactions and QT prolongation and is no longer approved in the US for NLC.
  - No approved drug alternative in use to treat NLC.

Conclusions

- FLX-787 has demonstrated a sigmoidal dose-response curve in a human EC model in vitro and in vivo in systemic exposure.
- Topical Chemical Neuro Stimulation of TRPV1/TRPA1 indirectly inhibits α-motor neuron hyperexcitability.
- FLX-787 has shown positive signals on cramp frequency in the parallel design portion of two exploratory human POC NLC studies.
- FLX-787 is well tolerated and safe, and no SAEs have been reported.
- Consistent with FDA guidance, future FLX-787 studies in NLC will be parallel design with emphasis on patient selection, data capture & monitoring.
- Clinical studies in MS and ALS are underway to explore the utility of FLX-787 in additional indications of different etiology where cramping and/or spasticity is prevalent.
- Planned initiation of IND-opening Phase 2 parallel design study in H1 2017.

References

- Lampl PM, Lomo T, Wulff CG. Neurons Decreases Muscle Cramps in Humans. Presentation Number: 537.15.
ORALLY INGESTED TRANSIENT RECEPTOR POTENTIAL (TRP) CHANNEL ACTIVATORS ATTENUATE THE INTENSITY-DURATION OF VOLUNTARILY INDUCED MUSCLE CRAMPS IN HUMANS

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Department of Kinesiology, Noll Laboratory, The Pennsylvania State University, University Park, PA 16802

ABSTRACT

Transient receptor potential (TRP) channels in the mouth, esophagus and stomach are activated by ingestion of many naturally occurring and synthetic compounds. Recent evidence demonstrates that TRP (channel) agonists may reflexly attenuate the intensity and/or duration of electically induced muscle cramps in humans1, presumably by decreasing motor neuron excitability. To test the effect of orally administered cell wall cramp agents, 35 subjects were randomly assigned to a double-blind, vehicle-controlled crossover study. Subjects performed 3 baseline experiments in which they underwent a voluntary exercise-induced muscle cramp protocol. From an original pool of 24 volunteers with a self-reported history of exercise-related muscle cramping, 6 men and 14 women (age 22±3 yrs representing 59% of screened subjects) were able to consistently reproduce cramps voluntarily. Each of the 20 enrolled subjects subsequently participated in 2 experimental trials, one 15 min after consuming less than 3 oz. of an active (A) formulation containing a mixture of TRPA1 and TRPV1 agonists derived from natural actives and another 15 min after ingesting a vehicle control (V). Subjects undertook an experiment on 2 separate days and were familiarized with the experimental protocol. An association was found between a positive subject’s history and the ability to consistently reproduce cramps (p<0.003). Subjects who had experienced the muscle cramps were able to produce a cramp in the pre-ingestion phase by pre-stretching the gastrocnemius, simulating the tibial surae, and then performing a maximal voluntary isometric contraction (MVC). Cramps were held for 30 s and a cramp occurred in 21 cramp subjects during the 10 min active attempt to elicit a cramp for a second time. Subjects repeated this pattern for up to 5 attempts within any given experiment; all experiments were separated by at least 1 week. EMG and force were recorded at baseline, during the active muscle contraction, and throughout the duration of the muscle cramp. There were no significant differences between active and vehicle control for pre-cramp MVCs (A 31.4±7.3% MVC; V 32.8±7.8% MVC; p>0.05); post-cramp MVCs (A 16.3±6.9% MVC; V 17.5±7.4% MVC; p>0.05); and cramp MVCs (A 10.9±4.6% MVC; V 13.7±6.4% MVC; p>0.05). However, the integrated EMG signal (i.e., the area under the EMG intensity-duration curve, below significantly lower in the A group (p<0.01) for the 20 minute (min) active period than in the V group (p<0.003). Comparative subjective ratings of soreness (on a 1 to 10 rating scale) during the initial 20 min after cessation of cramping were also significantly (p<0.02) lower for the active formulation (A 5.9±2.6) compared with results from studies that have evoked leg muscle cramps using electrical stimulation. Oral TRP channel activators significantly attenuated the intensity-duration profile of voluntarily induced muscle cramps in this sample of young adults.

METHODS

Recruitment and screening: 20 eligible volunteers were recruited for the trial, with 10 subjects on each treatment arm. Inclusion criteria included age (18-40 yrs), gender (M or F), BMI (21-28), and history of muscle cramps (~3 times per week). Exclusion criteria included history of chronic pain, neurological or orthopedic conditions, and contraindications for the study medications. Subjects were randomized to either A or V using a blinded computer-generated algorithm. Familiarization trials were conducted 48 h prior to the start of the study. The active treatment was a 2-ml dose containing 2 m of TRPV1 agonist (0.75 m of ALK-1025 in 0.75 m of PG; 80% of final concentration) and 1 m of TRPA1 agonist (20% of final concentration). The vehicle control was identical except for the omission of the TRP agonists. Subjects were familiarized with the protocol on 3 separate days. The familiarization protocol included the ingestion of a 2-ml placebo and the experience of a muscle cramp on each day, with the sequence of the familiarization protocol randomized to each subject. A total of 68 cramps were induced in 20 subjects over 3 familiarization days. The active treatment was administered 15 min prior to the commencement of the experiment. An experienced experimenter monitored for the occurrence of muscle cramps. When a cramp was detected, the experimenter terminated the voluntary cramp attempt and selected a new voluntary contraction in which the cramp was induced. The cramp was then assessed according to pre-established criteria.

RESULTS

The time to cramp onset was significantly lower in the active treatment (A 1.0±1.7 min) compared to the vehicle control (V 4.3±1.6 min; p<0.001). Cramps were significantly shorter (A 9.2±3.0 s) compared to the vehicle control (V 16.2±3.0 s; p<0.001). The area under the cramp intensity-duration curve was significantly lower in the active treatment (A 0.6±0.5; V 2.3±0.9; p<0.001). The soreness rating was significantly lower in the active treatment (A 2.1±1.4) compared to the vehicle control (V 7.2±1.2; p<0.001). Muscle cramps were significantly less intense and occurred less frequently in the active treatment group compared to the vehicle control group. Significant decreases were also observed for the intensity duration of the muscle cramp and the soreness rating at 20 min (p<0.001). The integrated EMG signal (i.e., the area under the EMG intensity-duration curve, below significantly lower in the A group (p<0.01) for the 20 minute (min) active period than in the V group (p<0.003). Comparative subjective ratings of soreness (on a 1 to 10 rating scale) during the initial 20 min after cessation of cramping were also significantly (p<0.02) lower for the active formulation (A 5.9±2.6) compared with results from studies that have evoked leg muscle cramps using electrical stimulation. Oral TRP channel activators significantly attenuated the intensity-duration profile of voluntarily induced muscle cramps in this sample of young adults.

SUMMARY AND CONCLUSIONS

• Oral consumption of a mixture of TRPV1 and TRPA1 agonists prior to exercise mitigated muscle cramp intensity and perceived soreness.
• Overall muscle activity during cramping (integrated area of EMG intensity-duration curve) was reduced with the active treatment.
• Self-reported ratings of muscle soreness were lower within the first 20 min after cramp cessation with the active treatment.
• No delay in cramp onset, shortening of cramp duration, or improved preservation of muscle force were detected with the active treatment.
• Athletes who experience EAMC may benefit from consuming TRP channel agonists immediately prior to exercise.

REFERENCES


FUNDING
Flex Pharma Inc.

IRB#2371
Contact: Daniel Craighed dhc139@psu.edu
Abstract

Purpose: Transient Receptor Potential (TRP) channel activation in the mouth, esophagus and stomach after ingestion of spicy food extracts can have direct effects on central nervous system (CNS) function that have been linked to increased maximal power output and decreased muscle cramps. However, no studies have evaluated the effects of consuming TRP agonists on exercise performance.

Methods: This “proof of concept” study was designed to test the effects of a spice-TRP channel activator drink (1.7 R oz with organic spice extracts, known TRPV1 and TRPA1 agonists; STA) on intermittent high-intensity cycling (Hi) using a randomized, double-blinded, placebo-controlled (PLA), crossover design in 10 healthy, active, college-aged men (n=10) and women (n=10). Subjects performed 2 trials (STA and PLA), each trial consisting of a 30 s maximal sprint (MS), 10 min rest, 45 min-MS (60% VO2max, ride with 1 min 100% VO2max, sprints every 5 min), 15 min rest, and a 10-min time trial (TT). Drinks were given before MS and TT. Performance measures included power output during MS (5-s intervals, mean, total), and distance covered during TT. Leg muscle pain (pain), heart rate, mean arterial pressure, body temperature, profile of mood state (mood), plasma glucose, IL-6, and IL-10 were also measured at multiple times during exercise and rest. Data were analyzed via paired t-tests and 2-way repeated-measures ANOVA.

Results: No significant differences (p<0.05) were found between STA and PLA for any of the variables. However, there was a consistent trend toward benefits of STA, including increased muscle power output (5 s intervals, mean, total, p=0.09), increased TT distance (13 of 19 subjects, p=0.20), reduced pain (p=0.17), and enhanced mood (p=0.20); all except TT distance covered during TT. Leg muscle pain (pain), heart rate, mean arterial pressure, core body temperature, profile of mood state (mood), plasma glucose, IL-6, and IL-10 were also measured at multiple times during exercise and rest. Data were analyzed via paired t-tests and 2-way repeated-measures ANOVA.

Conclusion: Results of this “proof of concept” study support further research on the CNS benefits of consuming natural spice-derived TRPV1 and TRPA1 agonists as a novel intervention to improve performance during intermittent high-intensity exercise, with no apparent adverse side effects.

Table 1. Participant characteristics

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Height* (cm)</th>
<th>Weight* (kg)</th>
<th>Body Fat* (%)</th>
<th>VO2max* (ml/kg/min)</th>
<th>VO2peak (L/min)</th>
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<tr>
<td>Male</td>
<td>23.9 ± 4.3</td>
<td>179.0 ± 7.8</td>
<td>84.0 ± 10.8</td>
<td>16.5 ± 5.4</td>
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<tr>
<td>Female</td>
<td>21.5 ± 1.8</td>
<td>164.4 ± 5.3</td>
<td>60.4 ± 6.5</td>
<td>24.4 ± 4.6</td>
<td>45.4 ± 5.2</td>
</tr>
</tbody>
</table>

*Values represented as mean ± standard deviation

Figure 1. Performance during 30-s maximal sprint

Figure 2. Performance during 10-min time trial

Figure 3. Leg muscle pain during experimental trial

Figure 4. Core body temperature during experimental trial

Figure 5. Mean arterial pressure during experimental visit

Conclusions

- There were no statistically significant differences between STA and PLA drinks on any of the variables measured in this study. However, there were consistent beneficial trends observed for STA treatment across several important variables including:
  - Greater mean power output (p=0.09) and total power output (p=0.09) during 30-s MS
  - Greater TT distance in 13 of 19 subjects (p=0.20)
  - Lower ratings of leg pain sensation during the course of experimental sessions (treatment*timepoint effect: p=0.17, Partial Eta2=0.07)
- No differences between treatments for cardiovascular, metabolic, or inflammatory measures.

These results support our central hypothesis of STA treatment working through a CNS mechanism of action.

These preliminary findings suggest that STA drink warrants further investigation as a novel dietary supplement to enhance exercise performance, with no apparent negative side effects.

Acknowledgements

The authors thank the participants and the staff of The STA Study. This study was supported by a grant from FlexPharma, Inc.
A MUSCLE CRAMP IS CAUSED BY HYPERACTIVE MOTOR NERVES.


A NUMBER OF FACTORS CAN, ALONE OR IN COMBINATION, INCREASE MOTOR NERVE EXCITABILITY.

AN ENHANCEMENT IN PERSISTENT INWARD CURRENTS (PICS), A NORMAL ASPECT OF NERVE FUNCTION AND COMMUNICATION, CAN RESULT IN MOTOR NERVE EXCITABILITY AND POTENTIALLY DRIVE CRAMPING.


THE INGREDIENTS IN HOTSHOT™ ACTIVATE TRANSIENT RECEPTOR POTENTIAL ION CHANNELS (TRPA1 AND TRPV1) THAT RESIDE IN THE MEMBRANES OF SENSORY NERVES IN THE OROPHARYNGEAL SPACE, SENDING NERVOUS IMPULSES FROM MOUTH TO BRAIN.


RESEARCH CONDUCTED ON HOTSHOT™ AND ITS TRP-ACTIVATING INGREDIENTS HAS DEMONSTRATED ITS IMPACT ON ATTENUATING MUSCLE CRAMPS. THE STIMULATION OF TRP CHANNELS ACTIVATES A NEURAL PATHWAY THAT RADIATES FROM THE MOUTH TO THE BRAIN, WITH ADDITIONAL NEURAL SIGNALS SENT DOWN THE SPINAL CORD THAT RETURN THE HYPERACTIVE MOTOR NERVES TO NORMAL FUNCTION, A RESPONSE THAT CAN BE EFFECTIVE AT PREVENTING AND STOPPING CRAMPING.


For more information, please email Info@TeamHOTSHOT.com.