TRPV1 and TRPA1 Activators Reduce Muscle Cramping: A Potential New Treatment for MS Symptoms

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Abstract

TRP-stim extract mixture decreases electrochemically-induced cramp intensity

TRP-stim extract mixture was analyzed by HPLC and peak retention times compared to known molecules present in the natural extract used to prepare the mixture. TRP-789 and TRP-781 showed HPLC 
profile evolutions with two known molecules. In comparison to FLX-788, FLX-787 was a minor component of the TRP-stim extract mixture.

Methods

Figure 2.

Background

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profile evolutions with two known molecules. In comparison to FLX-788, FLX-787 was a minor component of the TRP-stim extract mixture.

Figure 2. Muscle cramps were induced in the flexor hallucis brevis (FHB) muscle by electrical stimulation and monitored by EMG to quantify cramp intensity and duration. A stimulating electrode was placed on the sole of the foot distal to the abductor hallucis muscle and recording sensors over the FHB muscle (Figure 2). The subject’s medial plantar nerve was electrically stimulated by transcutaneous nerve stimulation (TNS) at above the experimentally determined threshold frequency to elicit a reproducible and robust cramp. The muscle cramp intensity and duration were evaluated and measured by EMG and quantified by calculating the area under the curve (AUC) and cramp duration post cessation of electrical stimulation (Figure 5). Muscle cramp intensity and duration were found to vary subject to subject, necessitating a pre-treatment EMG to serve as a subject-specific baseline control. After consumption of Flex Pharma product, measurement of FLX-787 and FLX-788 (Figure 5) showed an increase in the AUC and duration compared to baseline values. The time at which the subject received treatment or vehicle control was referred to as time point zero.

Figure 3. Schematic of human TRPV1 sub-type expression in the skin and associated sensory innervation. TRPV1 sub-type expression is shown in sensory nerves associated with the skin and subcutaneous area with red/orange colors to indicate high levels of expression. The areas of the skin are organized to reflect the sensory innervation patterns of the body: forearms (purple), upper arm (light purple), lower arm (blue), upper leg (light blue), lower leg (light orange), thigh (orange). Areas where sensory nerves are in contact with muscle cramping sites are outlined in red: right calf (red square), left calf (red square). The right core (red square) is the area of the right leg below the knee where muscle cramping is located. 

Figure 4. Schematic representation of human TRPA1 sub-type expression in the skin and associated sensory innervation. TRPA1 sub-type expression is shown in sensory nerves associated with the skin and subcutaneous area with red/orange colors to indicate high levels of expression. The areas of the skin are organized to reflect the sensory innervation patterns of the body: forearms (purple), upper arm (light purple), lower arm (blue), upper leg (light blue), lower leg (light orange), thigh (orange). Areas where sensory nerves are in contact with muscle cramping sites are outlined in red: right calf (red square), left calf (red square). The right core (red square) is the area of the right leg below the knee where muscle cramping is located. 

Results

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TRP-stim extract mixture was analyzed by HPLC and peak retention times compared to known molecules present in the natural extract used to prepare the mixture. TRP-789 and TRP-781 showed HPLC profile evolutions with two known molecules. In comparison to FLX-788, FLX-787 was a minor component of the TRP-stim extract mixture.

Figure 7. FLX-787 and FLX-788 were analyzed for the ability to activate other TRP or TRPV1. Both molecules were confirmed as agonists of the channels displaying similar potency. TRPV1 IC50s were determined by automated Patch-clamp (PattchClamp) in HEK cells stably transfected with human TRPV1. TRPA1 IC50's were measured by monitoring Col-Fura in CHO cells cotransfected with human TRPV1 pre-loaded with a calcium-sensitive fluorescence dye (DHP). 

Figure 8.

Conclusions

A mixture of natural extracts significantly inhibited the intensity and duration of electrically-induced cramps by 3-fold (p<0.0001) within minutes of injection, lasting up to 6-8 hours.

GMP-synthesized single agent molecules, FLX-787 and FLX-788, as well as double combinations of GMP-synthesized TRPV1 and TRPA1 agonists, FLX-787 and FLX-788, significantly decreased cramp intensity relative to vehicle (p<0.01).

FLX-787 and FLX-788 demonstrated improved efficacy at decreasing cramp intensity compared to the parental extract formulation by 2-fold.

Flex Pharma’s products were safe and well-tolerated across all studies.

Stimulation of TRPV1 and TRPA1 channels in the mucous membranes by one or two agonists is an effective strategy to inhibit cramping.

Chemical neuro stimulation may be a generally applicable method to treat disorders stemming from a-motor neuron hyperexcitability such as cramping or spas ticity due to ALS and MS.

Future Clinical Directions

• Multiple Sclerosis (MS) associated spasticity
  Between 210,000 and 350,000 people in the US suffer from MS, approximately 84% of whom experience spasticity.
  • Neuronal dysfunction associated with inflammatory and degenerative processes in the brain and spinal cord lead to hyperexcitable muscle cramping, causing contractions and spasticity.

Multi-Center MS Trial (EX-USA): A randomized, blinded, double cross-over study to investigate the effects of Flex clinical candidate in subjects with cramps, spasms and spasticity.

Resuscitation and monitoring of the patient (Diagnosticians of least concern) are not complete until a cramp is observed.

Nocturnal leg cramps (NLC)

50% or 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 are helpful.

• Quinine, prescribed in the United Kingdom for NLC, is associated with thrombo cytopenia, 
  hypersensitivity reactions and GI bleeding and is no longer approved in the US for NLC.

• No approved drug alternative in US to treat NLC.

Multi-Center Trial in NLC: A randomized, double-blind, placebo-controlled, double cross-over study to evaluate the effects of a Flex product on the frequency of nocturnal foot and/or leg cramps when self-administered approximately 45 minutes before going to bed.

Discussion

Brooke W. Hegarty is an employee of Flex Pharma; Glenn F. Short III is an employee of Flex Pharma; Bruce Bean is a stockholder and receives compensation from Flex Pharma. Christoph H. Westphal is a scientific advisory board member and board member; Thomas C. Wessel is an employee of Flex Pharma; Christoph H. Westphal is an employee of Flex Pharma; Jennifer M. Cermak is an employee of Flex Pharma.