Neuropeptides 48 (2014) 371–378

Contents lists available at ScienceDirect

Neuropeptides

journal homepage: www.elsevier.com/locate/npep

Analgesic topical capsaicinoid therapy increases somatostatin-like immunoreactivity in the human plasma

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ARTICLE INFO

Article history:
Received 28 May 2014
Accepted 7 October 2014
Available online 16 October 2014

Keywords:
Chronic low back pain
Capsaicinoid
Nonivamide
Somatostatin
TRPV1 receptor
Mechanism of action
Analgesic effect

ABSTRACT

The aim of the present study was to evaluate the therapeutic potential of local capsaicinoid (EMSPOMA® cream) treatment on chronic low back pain in patients with degenerative spine diseases and to investigate the possible mechanism of action of the therapy.

The qualitative and quantitative analyses of capsaicinoids in EMSPOMA® cream were performed by liquid chromatography–tandem mass spectrometry (LC–MS/MS). In the clinical study 20 patients with degenerative spine diseases were involved in a self-controlled examination. During the 21 day therapy they received 30 min daily treatment with capsaicinoid (EMSPOMA®) cream to the lumbar region of the back. The pain (VASs, Oswestry Disability Index) and the mobility of the lumbar region of the spine (Schober's, Domján's L and R test) were detected at baseline and at the end of the 1st, 2nd and 3rd weeks. The plasma level of somatostatin-like immunoreactivity (SST-LI) was measured by radioimmunoassay (RIA) before and after the treatment on the first and the last day of the therapy.

Nonivamide (0.01%) was identified as the only capsaicinoid molecule in the cream. In the clinical study the 21 day local nonivamide treatment reduced the pain sensation. Oswestry Disability Index decreased from 39 ± 3.9% to 32.5 ± 4.4%. VASs showed 37.29%–59.51% improvement. In the plasma level of SST-LI threefold elevation was observed after the first nonivamide treatment.

We conclude that nonivamide treatment exerts analgesic action in chronic low back pain and causes the release of the antinociceptive and anti-inflammatory neuropeptide somatostatin which may play pivotal role in the pain-relieving effect.

1. Introduction

The local capsaicinoid treatment is an old fashioned but still effective therapy in wide range of pain conditions including rheumatoid arthritis (Deal et al., 1991), osteoarthritis (Cameron et al., 2009), neuropathic pain (de Leon-Casasola, 2011) and soft tissue pain (Chrubasik et al., 2010). The chronic musculoskeletal disorders, back pain and arthritis influence negatively the quality of life (Sprangers et al., 2000) and cause high economic costs mainly because of the persistent inability to return to work due to the chronic pain (Gatchel et al., 1995; Yelin, 1998).

Non-steroidal anti-inflammatory drugs (NSAIDs) and COX-2 inhibitors with several adverse effects (Hawkey and Langman, 2003; Laine, 2003) are most commonly applied to relieve pain however sufficient evidence for NSAIDs efficacy on chronic low back pain is still lacking (Frerick et al., 2003).

Topical capsaicinoid treatments (creams, lotions and patches) with different concentrations of capsaicin (0.015–1%, 8%) have become widespread in inflammatory and arthritic conditions since the 1980s (Fernandes et al., 2013). Low-concentration capsicum patches have been described as an effective, analgesic, clinically relevant treatment in low back pain without systemic side effects compared to placebo controls (Frerick et al., 2003; Keitel et al., 2001). Capsaicin cream (0.015, 0.025, 0.075%) has significantly reduced pain in knee and hand osteoarthritis evaluated in multiple double-blind vehicle-controlled clinical trials (De Silva et al., 2010).

Most of the clinical studies investigate capsaicin or capsicum extracts. The effectiveness of nonivamide alone has not yet been examined in a clinical study (Weiser et al., 2013).

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http://dx.doi.org/10.1016/j.npep.2014.10.001
0143-4179/

Nonivamide (pelargonic acid vanillylamide, N-nonanoyl vanillylamide) a naturally occurring capsaicinoid in chili pepper (Kozukue et al., 2005; Reilly et al., 2001a) seemed to be less effective in several in vivo studies than capsaicin (Kawamura et al., 1993; Skofitsch et al., 1984). However, it was proved equipotent agonist of transient receptor potential vanilloid 1 (TRPV1) receptor investigated at the receptor level by voltage-clamp recordings using transfected cells (Weiser et al., 2013). TRPV1 is expressed on nociceptive nerve endings of unmyelinated C and small myelinated Aδ fibers. TRPV1 is non-selective cation channel which can be activated by exogenous and endogenous physical (heat) and chemical (low pH; vanilloid structures: capsaicin, resiniferatoxin, nonivamide) stimuli. Stimulation of the capsaicin-sensitive afferents causes local neurogenic inflammation (vasodilatation and plasma protein extravasation leading to edema formation) via the release of proinflammatory neuropeptides such as substance P (SP), neurokinin A and calcitonin gene-related peptide (CGRP) (Maggi, 1995; Pinter and Szolcsanyi, 1995; Szolcsanyi et al., 1998a). Besides these proinflammatory neuropeptides anti-inflammatory and analgesic neuropeptides such as somatostatin (SST) are also released from capsaicin-sensitive nerve endings and exerts systemic effects via the circulation (Szolcsányi, 2004; Szolcsanyi et al., 1998a, 1998b). SST acts on five (sst_{1-5}) G_i-protein coupled receptors which are expressed on variety of immune cells, vascular endothelial and smooth muscle cells, as well as neurons (Csaba and Dournaud, 2001; Pinter et al., 2006). The antinociceptive and anti-inflammatory actions of SST are mediated by sst_1 and sst_4 receptors (Helyes et al., 2001; Pinter et al., 2002, 2006; Szolcsanyi et al., 2004) but recently published data suggest that sst_2 receptor also plays role in the exertion of these effects of SST (Imhof et al., 2011; Shi et al., 2014). SST is able to elicit antinociceptive actions in acute (Szolcsanyi et al., 2004) and chronic (Bar et al., 2004; Helyes et al., 2004; Imhof et al., 2011) animal models. SST inhibits the release of proinflammatory mediators exerting immuno-regulatory function (Chowers et al., 2000; Elliott et al., 1999; Helyes et al., 1996, 2004; Szolcsanyi et al., 1998a) and reduces nociception by stimulation of peripheral and central neurons (Carlton et al., 2001; Helyes et al., 2000, 2004; Szolcsanyi et al., 1998b). SST and its receptors are expressed in the pain processing and regulatory pathways in the central nervous system (dorsal horn of cervical and lumbar spinal cord; motor neurons in ventral horn of spinal cord; spinal cord dorsal root ganglion; hypothalamus; thalamus; trigeminal sensory nucleus; cortex; hippocampus; striatum) and in the periphery (Kumar, 2009). SST inhibits the nociceptive dorsal horn neurons and exerts peripheral control on nociceptive input (Pinter et al., 2006).

The molecular mechanism of pain relieving effect of SST is not well understood yet. Activation of Gi-protein coupled SST receptors opens various K^+ channels and inhibits voltage gated Ca^{2+} channels (Koch et al., 1988) resulting inhibition of spike generation and release of neurotransmitters (Weckbecker et al., 2003), which processes play role in reduction of nociception.

SP, which is thought to be important signal for pain neurotransmission, is reduced in the skin after local capsaicin administration (Bernstein et al., 1981). Since 1980s these processes have been considered as the mechanism of action of topical capsaicin treatment, but reduction of SP content in the skin is rather a consequence of defunctionalization of capsaicin sensitive nerve terminals (Anand and Bley, 2011). Capsaicin is unable to diffuse into the deeper layer of the skin or to the joint, thus the defunctionalization cannot explain its analgesic effect in the case of musculoskeletal disorders (Anand and Bley, 2011).

It is supposed that release of SST from the capsaicin-sensitive nerve endings may play key role in the analgesic effect of local capsaicinoid treatment in joint diseases, and can be considered as a possible mechanism of action of the therapy. On the basis of our quantitative and qualitative analysis of the commercially available capsaicinoid cream EMSPOMA® by liquid chromatography–tandem mass spectrometry (LC–MS/MS) it has been found that nonivamide (0.01%) is the only capsaicinoid molecule in the cream. Therefore, we have investigated the effect of local nonivamide treatment in low back pain in patients with degenerative spine diseases, and we have detected the changes in SST plasma concentration during the therapy.

2. Materials and methods

2.1. Determination of capsaicinoids in EMSPOMA® cream

2.1.1. High pressure liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) system and method

The qualitative and quantitative analyses of capsaicinoids in EMSPOMA® cream were performed by LC–MS/MS using an Agilent 6530 Accurate Mass Q-TOF LC/MS system. The protonated molecules produced from capsaicin (m/z 306), dihydrocapsaicin (m/z 308) and nonivamide (m/z 294) were monitored. The Q-TOF MS instrument was operated with an Agilent Jet Stream ES ion source in positive ionization mode with a mass accuracy <1 ppm, a mass resolution of 10,000–20,000 (121–922 m/z), a measuring frequency of 10,000 transients/s and a detection frequency of 4 GHz. The Jet Stream ion source was operated using the following conditions: pressure of nebulizing gas (N2) was 25 psi, the temperature and flow rate of drying gas (N2) were 325 °C and 7 l/min, the temperature and flow rate of sheath gas were 300 °C and 10 l/min. The capillary voltage was 3000 V, fragmentor and skimmer potential was set to 125 V and 65 V, respectively. For the MS/MS analysis, collision-induced dissociation (CID) with a collision energy of 30 V was used. Reference ions used for mass calibration: purine 121.050873 $[M+H]^+$, HP-921 = hexakis (1*H*,1*H*,3*H*-tetrafluoropropoxy)phosphazine 922.009798 $[M+H]^+$. The measurements and post-run analyses were controlled by the software MassHunter Acquisition B.04.00 for the Agilent TOF and QTOF.

We used the validated chromatographic method developed by Kaale et al. (2002) to separate and quantitate capsaicinoids extracted from a topical cream. The chromatographic separations were performed at 30 °C on a Zorbax Extend C18 column (50 mm × 2.1 mm, 1.8 μm, Agilent, USA). Two mobile phases, solvent A, which consisted of 0.1 v/v% formic acid in water and solvent B, 0.1 v/v% formic acid in methanol were used. The gradient profile was set as follows: 0.00 min 50% B eluent, 5.5 min 100% B eluent. The flow rate was 0.2 ml/min, the injection volume was 1 μl.

2.1.2. Preparation of sample

Sample preparation was performed according to the extraction method developed by Kaale et al. (2002) for the extraction of capsaicinoids from a topical cream. In their experiments recoveries of more than 95% were achieved with R.S.D. values between 0.6 and 1.3%. Into a 100 ml conical flask, 0.5051 g of the EMSPOMA® cream was weighed and 20.0 ml of methanol– water (80:20) and 20.0 ml of hexane were added. The mixture was thoroughly mixed for 10 min to make a uniform emulsion. The emulsion was centrifuged at 3580 *g* for 20 min and 15.0 ml of the lower aqueous layer was pipetted into a 50.0 ml volumetric flask. The extraction procedure was repeated twice by adding each time 15.0 ml of methanol–water (80:20) and withdrawing the same volume. This makes a total volume of 45 ml, which was replenished to 50.0 ml using the same diluent. We made a 50% dilution of this solution and injected to the column.

2.1.3. Preparation of quality control solution

Standard stock solution: capsaicin (0.5 mg), dihydrocapsaicin (0.5 mg) and nonivamide (0.5 mg) were solved in 10 ml methanol–water (80:20). This stock solution was used to prepare the quality control solution (0.5 μg/ml).