Effects of Topical Capsaicin on Cutaneous Innervation: Implications for Pain Management

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Abstract: Changes in cutaneous innervation are a hallmark of neuropathic pain syndromes. Although in few cases the density of cutaneous innervation increases in painful areas, for the most part the density of nociceptive sensory nerve endings decreases and the degree of deinnervation seems to correlate with the severity of pain. In combination with tests for nociceptor function, immunostaining for protein gene product 9.5 (PGP 9.5) is commonly used as diagnostic tool to indicate these pathophysiological changes in cutaneous innervation. However, sole reliance on PGP 9.5 may underestimate the presence of regenerating sensory nerve terminals or fail to capture changes in the expression of proteins such as ion channels or receptors. Topical capsaicin consistently reduces intra-epidermal nerve fiber density assayed via PGP 9.5, and also increases thresholds for activation of some thermoreceptors. The mechanism of action involves a highly localized insult to cutaneous nociceptors mediated by activation of TRPV1 and calcium overload, and perhaps even a direct toxicity to mitochondria. It is possible that topical capsaicin and lidocaine share an ability to reduce cutaneous innervation by inducing localized toxicity in mitochondria-rich nociceptive terminals. Overall, the high local concentrations of drugs and even excipients delivered by topical analgesics into the skin may be able to activate secondary pharmacodynamic processes. Optimizing topical formulations of capsaicin or other analgesics to maximize pain relief with the fewest adverse effects is not a simple matter of varying drug concentration, and it is highly questionable whether ‘bioequivalence’ could ever be based simply upon equivalent cutaneous drug delivery.

Keywords: Capsaicin, nociceptor, pain, PGP 9.5, lidocaine, TRPV1.

INTRODUCTION

Capsaicin has a long history of human use as a food additive and medicine, and has been the subject of thousands of research papers. The first formal report of the pain-reducing properties of topical capsaicin appeared in 1850 as a recommendation to use an alcoholic hot pepper extract on burning or itching extremities [1]. Creams, lotions and patches containing capsaicin, generally in the range of 0.025 to 0.1% by weight, are now available in many countries, often without the requirement of a prescription, for the management of neuropathic and musculoskeletal pain. Clinical studies involving three to five topical skin applications per day for periods of 2 to 6 weeks have generally suggested modest benefits against various pain syndromes, including osteoarthritis, postherpetic neuralgia, and painful diabetic neuropathy [2, 3]. With the recent FDA and EMA approvals of Qutenza®, a single-use high-concentration topical capsaicin formulation for the management of peripheral neuropathic pain, substantially more formal data on this drug substance is now available.

Capsaicin and its topical delivery have frequently been reviewed [e.g., 4, 5]. This article addresses some of the knowledge gaps which have yet to be addressed in previous literature, and summarizes some of the key data pointing to potential indications for topical capsaicin. First, is an examination of the ways that innervation of the skin – the site of action – have been studied and a survey of extensive literature on changes in cutaneous innervation. Attention is then turned to the effects of capsaicin, its dominant mechanism of action and the possibility that a contribution from secondary pharmacological mechanisms cannot be excluded. It will be suggested that capsaicin may have shared mechanisms of action with another topical analgesic. Finally, the limitations of focusing merely on capsaicin concentrations and not the delivery rate and other potentially neurotoxic components of topical formulations are discussed.

CHANGES IN CUTANEOUS INNERVATION WITH PAIN SYNDROMES AND CAPSAICIN EXPOSURES

The somatosensory nervous system undergoes integrated responses and adaptations to a wide variety of traumas, diseases and metabolic or iatrogenic insults. The most peripheral aspects of the sensory nervous system may display the most substantial changes, as sensory nerve fibers and terminals in the epidermis and dermis are both highly vulnerable to superficial trauma and completely reliant upon the health and integrity of their supportive cell bodies and proximal axons. Because intact cutaneous innervation is dependent upon so many factors, innervation of the skin is somewhat heterogeneous in healthy young adults and becomes progressively more heterogeneous with aging due to cumulative environmental and internal factors [6].
Changes in innervation of the epidermis and dermis by sensory nerve fibers can be quantified by two functional assays: quantitative sensory testing (QST) and laser-evoked potentials (LEPs). QST involves analysis of responses to stimuli of controlled intensity. Responses to thermal stimuli can be quantified by applying probes which are rapidly heated or cooled and activate cutaneous thermoreceptors [7]. Mechanical sensitivity to tactile stimuli can be measured by using von Frey hairs or monofilaments, pricking with pins and electronic vibrometers. LEPs are measurements of cortical responses using lasers to selectively stimulate various thermoreceptors [8]. Lasers can emit radiant-heat pulses which – in some protocols – selectively activate the two classes of cutaneous nociceptors (Aδ- and C-fiber nerve endings). When performed by skilled operators, QST and LEP exams have high sensitivity and are very reliable in assessing damage to both peripheral and central nervous systems. However, both QST- and LEP-based examinations are time consuming and rely upon expensive equipment. Moreover – especially for QST – training of the operator (and even the subjects) and highly consistent techniques are critical to obtaining reliable and highly reproducible data [9]. Therefore, QST and LEP are not best suited for quantitative comparison of cutaneous innervation data across studies.

A method much less reliant on the clinical skill of the operator and thus more suitable for data pooling across laboratories involves quantification of cutaneous innervation via skin punch biopsies (often 3 mm in diameter). Skin punch biopsies constitute a relatively easy procedure and are minimally invasive [10]. Tissue samples are first preserved and then stained for various antigens present in the cutaneous nociceptors. The most commonly used antibody is to protein gene product 9.5 (PGP 9.5), which is also known as ubiquitin carboxyl terminal hydrolase-1 [11]. Using counting rules that measure the number of nerve fibers which cross the dermal-epidermal junction, the density of intra-epidermal nerve fibers (IENFs) is obtained [12]. As it is widely assumed that all nerve fibers present in the epidermis are nociceptors (C- and some Aδ-fibers), IENF density is thought to be reflective of nociceptor innervation. Although initially available in only a few research centers, 3-mm punch biopsies for PGP 9.5 analysis are now much more widely available and can be ordered by many physicians as a diagnostic tool. Accordingly, substantially more data on cutaneous innervation is available from punch biopsies than from functional evaluations.

There are several important limitations to information gleaned from PGP 9.5 immunostaining, the first of which relates to the surprisingly wide distribution of the protein. Although PGP 9.5 expression in normal tissues was originally felt to be a structural component present in mature nerve fibers, strictly confined to neurons and neuroendocrine cells, PGP 9.5 has been subsequently reported in distal renal tubular epithelium, spermatogonia, Leydig cells, oocytes, melanocytes, prostatic secretory epithelium, ejaculatory duct cells, epididymis, mammary epithelial cells, Merkel cells and dermal fibroblasts [13]. Fortunately, few of these non-neuronal cell types found in the epidermis and thus are unlikely to diminish the reliability of IENF density analyses. However, intra-observer variability may be caused by differences in counting rules or their interpretation, or a basement membrane that is poorly visualized [14]. Another limitation for the study of changes in cutaneous innervation with diseases or trauma follows from the nature of PGP 9.5, which is a ‘housekeeping’ or structural protein present in mature nerve terminals. Several reports show that PGP 9.5 may be absent from growing nerve fibers that can be visualized by other markers. For instance, several studies have shown that regenerating nerve fibers may express antigens like growth-associated protein 43 (GAP-43) before PGP 9.5 [15]. In a study of the time-course of recovery of LEPs following topical capsaicin treatment, functional recovery occurred before PGP 9.5 immunostaining occurred, but fibers marked by GAP-43 did show a good correlation with the functional responses [16]. Another recent study further points to the complexity of the relationship between these two markers, as the correlation between PGP 9.5 and GAP-43 expression was diminished in diabetic patients relative to normal controls [17]. Finally, perhaps the most problematic limitation of PGP 9.5 analyses was introduced above: cutaneous innervation is somewhat heterogeneous in healthy young people, becomes more heterogeneous with age, and significantly more heterogeneous with metabolic disease or damage to the sensory nervous system. Therefore, the prognostic value (the ‘signal-to-noise ratio’) may be limited when based on a single 3-mm punch biopsy taken from a large area of painful involvement.

Even with all of these limitations, the best poolable information available about changes in cutaneous innervation relies upon data generated with PGP 9.5. Using this antigen, alterations of IENF density has been observed in many types of neuropathic pain and other syndromes or diseases. Table 1 shows the correlations between peripheral neuropathies, diseases and other disorders and changes in PGP 9.5 immunostaining. The degree of IENF reductions appears to correlate with the severity of pains or disorders. As has been articulated elsewhere [5, 18, 19], it is the hyperactivity or hypersensitivity of residual cutaneous nociceptors that is thought to provide the sensory barriers that drive many chronic pain syndromes.

Administration of topical capsaicin causes a reduction in cutaneous nociceptor function and innervation. Many immunohistochemical studies using antibodies to PGP 9.5 or other nerve fiber proteins provide evidence that capsaicin can produce highly localized loss of nociceptive nerve fiber terminals in the epidermis and dermis [20] (see Fig. 1). Nociceptors reappear – using PGP 9.5 visualization – over the course of weeks. In the case of Qutenza-induced deinnervation, essentially complete recovery is observed by 24 weeks [21]. Table 2 summarizes available data regarding these effects of topical capsaicin.

**TOPICAL CAPSAICIN’S MECHANISMS OF ACTION IN THE SKIN**

The reduction of cutaneous innervation following topical administration of capsaicin may involve a convergent insult on mitochondria. The pharmacological activities of capsaicin and its primary receptor have been widely reviewed [e.g., 22], so only summary information is presented here. Capsaicin is a highly selective and potent (low nM affinity) exogenous agonist for the transient receptor potential vanilloid 1 (TRPV1) receptor, a trans-membrane receptor-ion channel complex which provides integrated responses to
Table 1. Survey of changes in cutaneous innervation with peripheral neuropathies, diseases and other disorders assayed primarily by changes in epidermal immunostaining for PGP 9.5 but sometimes other antigens. Values for IENF alterations are derived from comparisons between affected areas and non-painful/unaffected skin or healthy subjects; many of these values were calculated based on other values provided in publications, therefore they should be considered useful approximations but not statistically rigorous. Changes reported in non-cornified skin or mucosal membranes are omitted, as are cancer- or tumor-related alterations.

<table>
<thead>
<tr>
<th>Pain Syndrome, Disorder or Disease</th>
<th>IENF Alteration</th>
<th>Key Findings</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer chemotherapy</td>
<td>39% reduction at 360 days</td>
<td>Significant reduction in distal leg (but not thigh) IENF density in patients receiving oxaliplatin. IENF loss continued to worsen following discontinuation of oxaliplatin</td>
<td>[64]</td>
</tr>
<tr>
<td>Celiac disease</td>
<td>Reduced in 5 of 8 patients</td>
<td>Neuropathies were diagnosed before celiac disease</td>
<td>[66]</td>
</tr>
<tr>
<td>Complex regional pain syndrome-1</td>
<td>71% reduction relative to contralateral control sites</td>
<td>Diminished at the CRPS-affected sites of 17/18 subjects</td>
<td>[67]</td>
</tr>
<tr>
<td>Diabetes – pancreas transplantation</td>
<td>96% reduction in calf, 92% in thigh</td>
<td>At one month post-transplant for type 1 diabetes, no evidence of regeneration</td>
<td>[68]</td>
</tr>
<tr>
<td>Diabetic neuropathy &amp; painful diabetic neuropathy</td>
<td>Reduction highly significant</td>
<td>Consistent reduction of epidermal and dermal PGP 9.5 and CGRP fibers</td>
<td>[70]</td>
</tr>
<tr>
<td>Diabetic truncal neuropathy</td>
<td>Clear reduction in all 3 patients</td>
<td>Positive correlation extent IENF reduction and pain severity</td>
<td>[76]</td>
</tr>
<tr>
<td>Fabry disease</td>
<td>74% reduction</td>
<td>All patients had severe loss of IENFs at ankle, but loss at distal thigh was less severe</td>
<td>[77]</td>
</tr>
<tr>
<td>Reduced at baseline</td>
<td>With 6 months of enzyme replacement therapy, no evidence of epidermal reinnervation</td>
<td></td>
<td>[78]</td>
</tr>
<tr>
<td>Reduced at fingertip (77%), thigh (51%), and leg (53%)</td>
<td>IENFs showed morphological abnormalities such as axonal varicosities or swelling. Proximal-distal gradient of ENFD in patients</td>
<td></td>
<td>[79]</td>
</tr>
<tr>
<td>Guillian-Barré syndrome</td>
<td>50% reduction</td>
<td>55% of patients had degeneration in the dermis, indicated by fragmentation sub-epidermal nerve plexuses and beaded appearance</td>
<td>[81]</td>
</tr>
<tr>
<td>HIV-peripheral neuropathy</td>
<td>Reduced</td>
<td>IENF density inversely correlated with neuropathic pain as measured by patients and physician global pain assessments. Decreased IENF density at distal leg associated with lower CD4 counts and higher plasma HIV RNA levels</td>
<td>[83]</td>
</tr>
</tbody>
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Table 1 contd…

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<thead>
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<tbody>
<tr>
<td>Reduced</td>
<td>ENFD reductions correlated with clinical and electrophysiologic severity of distal sensory polyneuropathy</td>
<td>[84]</td>
<td></td>
</tr>
<tr>
<td>56% decrease</td>
<td>ENFD at the distal leg was lower in symptomatic neuropathy but not asymptomatic neuropathy group compared to neuropathy-free patients</td>
<td>[85]</td>
<td></td>
</tr>
<tr>
<td>Leprosy</td>
<td>Pain-related evoked potential latencies and amplitudes strongly correlated with reductions in IENF density</td>
<td>[86]</td>
<td></td>
</tr>
<tr>
<td>73% reduction in most severely affected skin</td>
<td>Reduction in IENFs correlated with loss of axon reflex vasodilation induced by capsaicin</td>
<td>[87]</td>
<td></td>
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<tr>
<td>Lupus erythematosus</td>
<td>Decrease; not quantified</td>
<td>[88]</td>
<td></td>
</tr>
<tr>
<td>63% reduction</td>
<td>PGP 9.5- and neurofilament-immunoreactive nerve fibers present in early type, but reduced with leprosy regression</td>
<td>[89]</td>
<td></td>
</tr>
<tr>
<td>40% reduction</td>
<td>Patients with active lupus had lower IENF densities than those with quiescent lupus</td>
<td>[90]</td>
<td></td>
</tr>
<tr>
<td>Metabolic syndrome</td>
<td>Reduction of epidermal nerve fiber length</td>
<td>[91]</td>
<td></td>
</tr>
<tr>
<td>Notalgia paresthetica</td>
<td>Epidermal nerve length was reduced and correlated negatively with sensory symptoms, signs, HDL-C, and sural nerve amplitudes</td>
<td>[92]</td>
<td></td>
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<tr>
<td>Parkinson's disease</td>
<td>No change in neuropeptide expression</td>
<td>[93]</td>
<td></td>
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<tr>
<td>Peripheral nerve injury – lower limb</td>
<td>Epidermal dendritic cells (possibly Langerhans) significantly increased</td>
<td>[94]</td>
<td></td>
</tr>
<tr>
<td>Postherpetic neuralgia</td>
<td>No change in neuropeptide expression</td>
<td>[95]</td>
<td></td>
</tr>
<tr>
<td>80% reduction</td>
<td>Patients showed increased in tactile and thermal thresholds and reductions in mechanical pain perceptions</td>
<td>[96]</td>
<td></td>
</tr>
<tr>
<td>77% reduction</td>
<td>Density of Meissner corpuscles also reduced</td>
<td>[97]</td>
<td></td>
</tr>
<tr>
<td>40% reduction</td>
<td>Evidence of nerve remodeling, such as irregular distribution along the epidermis and an increase in nerve branching</td>
<td>[98]</td>
<td></td>
</tr>
<tr>
<td>Post-surgical neuropathy – hand</td>
<td>Sensory profile of affected area was general deficit of thermal, tactile, and pain sensitivity, only plus symptom was hyperalgesia to pressure threshold</td>
<td>[99]</td>
<td></td>
</tr>
<tr>
<td>55% decrease in non-pain patients, 31% decrease in pain patients</td>
<td>Only in the distal leg was significant decrease in IENF of patients compared to controls</td>
<td>[100]</td>
<td></td>
</tr>
<tr>
<td>Post-surgical neuropathy – breast</td>
<td>No pattern of subjective, functional or structural parameters separated neuropathy patients into pain and non-pain patients</td>
<td>[101]</td>
<td></td>
</tr>
<tr>
<td>Sarcoïdosis</td>
<td>Nerve growth factor present in basal and some supra-basal keratinocytes</td>
<td>[102]</td>
<td></td>
</tr>
<tr>
<td>Reduced, or normal density but abnormal morphology</td>
<td>TRPV3 and TRPV4 significantly increased in keratinocytes in breast pain tissues</td>
<td>[103]</td>
<td></td>
</tr>
<tr>
<td>26% reduction</td>
<td>Lower IENF values in men, and an age-related decrease in both genders</td>
<td>[104]</td>
<td></td>
</tr>
<tr>
<td>Small-fiber neuropathy</td>
<td>Reduced</td>
<td>[105]</td>
<td></td>
</tr>
<tr>
<td>86% reduction</td>
<td>Counting IENFs morphometrically per epidermal area or per epidermal length yields similar results</td>
<td>[106]</td>
<td></td>
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</tbody>
</table>
Table 1. contd…

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</thead>
<tbody>
<tr>
<td>Reduced in 71% of patients</td>
<td>Identified associations included abnormal glucose metabolism, Lyme vaccination, monoclonal gammopathy, vitamin B12 deficiency, coeliac disease, and diseases of the connective tissue, inflammatory bowel and thyroid; etiology undetermined in 50% of patients</td>
<td>[105]</td>
<td></td>
</tr>
<tr>
<td>Significantly lower (labeled by TRPV1 and PGP 9.5) in painful neuropathies, at both proximal region of the thigh and distal region of the leg</td>
<td>Densities obtained with TRPV1 antibody did not differ from those with PGP 9.5 and anti-unique β-tubulin</td>
<td>[106]</td>
<td></td>
</tr>
<tr>
<td>Vulvodynia</td>
<td>Increased</td>
<td>Intraepithelial nerve fiber profiles were not evenly distributed; some areas were almost void of nerves, whereas other parts were densely innervated</td>
<td>[107]</td>
</tr>
<tr>
<td></td>
<td>57% increased in area and 60% increase in density</td>
<td>No change in PGP 9.5-immunoreactive nerves in the deep dermis, especially around the blood vessels and sweat glands</td>
<td>[108]</td>
</tr>
<tr>
<td></td>
<td>Increased</td>
<td>Significant increase of TRPV1 fibers in sub-epidermis of patients</td>
<td>[109]</td>
</tr>
</tbody>
</table>

![Fig. (1). Visualization of IENFs and time course of Qutenza-induced effects. A) Representative images of intra-epidermal nerve fiber (IENF) density in skin areas exposed to Qutenza for 60 minutes, and in unexposed control areas on the thighs of healthy volunteers. Immunoreactivity to PGP 9.5 is yellow or green, Type IV Collagen is red, and the epidermis is blue. B) Mean reductions in IENF density at 1, 12 and 24 weeks after a single Qutenza patch application. *indicates P < .05 relative to control. Slightly modified from Kennedy et al., 2010.](image)

Temperature, pH and endogenous lipids. When activated by a combination of heat, acidosis or endogenous/exogenous agonists, TRPV1 opens transiently and initiates a depolarization mediated by influx of sodium and calcium ions. In the nociceptive (mostly C- and some Aδ-fibers) nerve endings which selectively express TRPV1, capsaicin-mediated depolarization results in action potentials, which are transmitted to the spinal cord and brain and usually reported as warming, burning, stinging or itching sensations (see Fig. 2).

In contrast to the transient physiological activation of nociceptors which arises from normal environmental stimuli or tissue injury, activation of TRPV1-expressing nerve fibers by capsaicin can be followed by effects on nociceptor functionality and structural integrity [5, 23]. Acutely, inactivation of voltage-dependent sodium channels will reduce electrical excitability, and is often accompanied by the osmotic swelling due to the chloride accumulation that accompanies influxes of positively charged ions [23]. Importantly for persistent effects, the TRPV1 channel is
Table 2. Effects of capsaicin on IENF density

<table>
<thead>
<tr>
<th>Drug or Treatment</th>
<th>Concentration or Dose; Pretreatment</th>
<th>Total Dose</th>
<th>Duration &amp; Frequency Exposure</th>
<th>Delivery Site &amp; Method</th>
<th>Subjects</th>
<th>IENF Reduction &amp; Time Course</th>
<th>ENFD Recovery</th>
<th>Deeper Effects &amp; Other Info</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qutenza 8% w/w patch; no topical lidocaine</td>
<td>3 sites, 3 x 3 cm, (640 µg/cm²)</td>
<td>Single 30, 60 &amp; 120 min</td>
<td>Inner upper thighs</td>
<td>Healthy</td>
<td>~59% (60 min), 63% (90 min); at 7 days</td>
<td>N.D.</td>
<td>Dermal effects not quantified</td>
<td>[48]</td>
<td></td>
</tr>
<tr>
<td>Qutenza 8% w/w patch; no topical lidocaine</td>
<td>3 sites, 5 x 5.5 cm, (640 µg/cm²)</td>
<td>Single 60 min</td>
<td>Inner upper thighs</td>
<td>Healthy</td>
<td>~80% (7 days), 20% (12 wk), 7% (24 wk)</td>
<td>80% recovery 12 wk, 93% at 24 wk</td>
<td>Reduction in dermis, not quantified</td>
<td>[21]</td>
<td></td>
</tr>
<tr>
<td>Qutenza 8% w/w patch; 30 min 4% lidocaine</td>
<td>1 site</td>
<td>Single 60 min</td>
<td>Inner upper thighs</td>
<td>Healthy</td>
<td>~71% at 7 days</td>
<td>N.D.</td>
<td>N.D.</td>
<td>[109]</td>
<td></td>
</tr>
<tr>
<td>NGX-1998 10% w/v; 30 min 4% lidocaine</td>
<td>3 sites, 5 x 5 cm, ~6 µL/cm²</td>
<td>Single 5, 15 &amp; 25 min</td>
<td>Inner upper thighs</td>
<td>Healthy</td>
<td>70% (5 &amp; 15 min) &amp; 73% (25 min) at 7 days</td>
<td>N.D.</td>
<td>N.D.</td>
<td>[109]</td>
<td></td>
</tr>
<tr>
<td>NGX-1998 &amp; NGX-4162 10% w/v; no topical lidocaine</td>
<td>3 sites, 5 x 5 cm, 15 µL/cm²</td>
<td>Single 15 min</td>
<td>Inner upper thighs</td>
<td>Healthy</td>
<td>34% (NGX-1998) &amp; 33% (NGX-4162) at 7 days</td>
<td>N.D.</td>
<td>N.D.</td>
<td>[110]</td>
<td></td>
</tr>
<tr>
<td>Capsaicin 0.1% cream</td>
<td>2 X 1800 mg</td>
<td>48 hr (2 X 24 hr)</td>
<td>Distal lateral thigh; Under occlusion</td>
<td>Healthy &amp; Diabetic</td>
<td>&gt; 99%</td>
<td>Rate reinnervation 0.18 (healthy), 0.07 (diabetics) fibers/mm/day; Diabetics 42% of healthy; Incomplete regrowth at 100 days</td>
<td>Rate reinnervation 0.17 (healthy), 0.13 (HIV+), 0.10 (HIV-PN) fibers/mm/day</td>
<td>Subepidermal-dermal plexus deinnervated</td>
<td>[46]</td>
</tr>
<tr>
<td>Capsaicin 0.1% cream</td>
<td>2 X 1800 mg</td>
<td>48 hr (2 X 24 hr)</td>
<td>Distal lateral thigh; Under occlusion</td>
<td>HIV-infected &amp; Healthy</td>
<td>&gt; 99%</td>
<td>Rate reinnervation 0.19 (healthy), 0.09 (diabetes) fibers/mm/day</td>
<td>Rate reinnervation 0.19 (healthy), 0.09 (diabetes) fibers/mm/day</td>
<td>Subepidermal-dermal plexus deinnervated</td>
<td>[73]</td>
</tr>
<tr>
<td>Capsaicin 0.1% cream</td>
<td>2 X 1800 mg</td>
<td>48 hr (2 X 24 hr)</td>
<td>Volar forearm; Under occlusion</td>
<td>Healthy</td>
<td>&gt; 99%</td>
<td>150 days for full regeneration</td>
<td>Reduction in sudomotor, vasomotor, pilomotor, and sensory function</td>
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<td>[47]</td>
</tr>
<tr>
<td>Capsaicin 0.1% cream</td>
<td>2 X 1800 mg</td>
<td>48 hr (2 X 24 hr)</td>
<td>Distal lateral thigh; Under occlusion</td>
<td>Diabetic &amp; Healthy</td>
<td>&gt; 99%</td>
<td>Rate reinnervation 0.19 (healthy), 0.09 (diabetic) fibers/mm/day</td>
<td>Rate reinnervation 0.19 (healthy), 0.09 (diabetic) fibers/mm/day</td>
<td>GAP-43 staining not extend into epidermis</td>
<td>[112]</td>
</tr>
<tr>
<td>Capsaicin Injection 0.2, 2 or 20 µg</td>
<td>5 mm area</td>
<td>Up to 7 injections</td>
<td>Lateral aspect upper arm (shoulder)</td>
<td>Healthy</td>
<td>~100% at 72 hr for 2 or 20 µg</td>
<td>&lt; 50% at 6 wk</td>
<td>Small to moderate decrease subepidermal plexus</td>
<td>Small to moderate decrease subepidermal plexus</td>
<td>[113]</td>
</tr>
<tr>
<td>Capsaicin 0.075% cream</td>
<td>35 cm²</td>
<td>4 times daily for 3 wk</td>
<td>Inner arm or volar forearm</td>
<td>Healthy</td>
<td>82% at 3 wk</td>
<td>After 6 wk, 88% recovery</td>
<td>Little effect subepidermal plexus or dermis</td>
<td>Little effect subepidermal plexus or dermis</td>
<td>[20]</td>
</tr>
<tr>
<td>Capsaicin 0.075% cream</td>
<td>4 x 5 cm</td>
<td>4 times day for 7 days</td>
<td>Volar forearm</td>
<td>Healthy</td>
<td>98% at 6 days</td>
<td>99% at 6 days</td>
<td>No effect subepidermal plexus or dermis</td>
<td>No effect subepidermal plexus or dermis</td>
<td>[114]</td>
</tr>
<tr>
<td>Capsaicin 0.075% dried on disk, applied</td>
<td>2.5 cm diameter</td>
<td>Single 5-minute application (%)</td>
<td>Volar forearm</td>
<td>Healthy</td>
<td>Significant reduction PGP 9.5, complete loss sub P</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>[115]</td>
</tr>
<tr>
<td>Capsaicin 0.075% cream</td>
<td>3.5 gm cream under occlusion, 5 x 5 cm</td>
<td>3 times for 24 hr</td>
<td>Lateral distal calf</td>
<td>Healthy</td>
<td>93% at end of dosing (72 hr)</td>
<td>Recover to 40% at 26 days; 56% at 54 days</td>
<td>GAG-43 staining: less reduction, much faster return, little in epidermis</td>
<td>GAG-43 staining: less reduction, much faster return, little in epidermis</td>
<td>[16]</td>
</tr>
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Fig. (2). Activation of TRPV1 by capsaicin results in sensory neuronal depolarization, and can induce local sensitization to activation by heat, acidosis, and endogenous agonists. Topical exposure to capsaicin leads to the sensations of heat, burning, stinging, or itching. High concentrations of capsaicin or repeated applications can produce a persistent local effect on cutaneous nociceptors, which is best described as ‘defunctionalization’ and constituted by reduced spontaneous activity and a loss of responsiveness to a wide range of sensory stimuli. Taken from Anand and Bley, 2011.

highly calcium permeable (with a calcium:sodium permeability ratio that is as high as 25:1) [24], which can produce dramatic increases in intracellular calcium. TRPV1 is also expressed on the endoplasmic reticulum, so capsaicin can additionally cause an intracellular release of calcium [25]. High intracellular calcium can additionally induce release from internal stores via calcium-dependent calcium release [26]. These multiple sources of calcium can overwhelm local calcium sequestration mechanisms, thereby activating calcium-dependent enzymes such as proteases [27] and inducing depolymerization of cytoskeletal components such as microtubules [28, 29].

Mitochondria are not randomly distributed throughout the sensory neuron but rather accumulate at regions of increased energy consumption, for example, at the sites of sensory transduction [30]. Numerous mitochondria are present in the peripheral terminals of nociceptors and may congregate there in response to nerve growth factor signaling. The primary insult to nociceptor function from capsaicin exposure is a robust intracellular calcium signal, which is sometimes sufficient to overload the calcium sequestration capabilities of mitochondria [23]. In addition, it is possible that topical capsaicin administration – particularly from high-concentration formulations such as Qutenza – may provide sufficiently high cutaneous capsaicin concentrations to enable secondary pharmacodynamic mechanisms to contribute to long-term effects on nociceptors. As observed in the context of the anti-cancer effects of capsaicin, TRPV1-independent effects may involve direct inhibition of mitochondrial respiration [31]. At concentrations much higher than required to activate TRPV1, capsaicin can compete with ubiquinone to inhibit directly electron chain transport [32]. Consequently, capsaicin may directly dissipate mitochondrial trans-membrane potential [33]. The EC$_{50}$ value for capsaicin to depolarize mitochondria in sensory neurons is 6.9 µM, a concentration much greater than required to activate TRPV1 [34]; it is not known whether this high concentration reflects a contribution from a direct effect on mitochondrial respiration.

Loss of mitochondrial function due to calcium overload and possible inhibition of metabolism may render affected nerve processes unable to maintain plasma membrane integrity and thus cause collapse of nerve endings to the depth where the capsaicin exposure was insufficient to irreversibly overwhelm mitochondrial function (see Fig. 3). If nerve fibers in skin retract or degenerate to the depth at which mitochondrial function was preserved, it is expected that markers for any constituent of those fibers will show reductions. However, it is important to recognize that loss of function and nerve terminal degeneration are distinct phenomena, each with potentially different time courses. In the simplest case, a loss of electrical excitability may occur via depolarization block or sodium channel inactivation, independently of a loss of axonal integrity. Other important physiological roles such as fast axonal transport of growth factors can also be compromised by capsaicin without axonal collapse [35]. Even though a general parallel between capsaicin-induced functional and structural changes is expected, there may be differences in the time courses.

The term ‘desensitization’ is often used to describe both the acute and persistent effects of capsaicin on sensory nerve function, but is unsatisfactory in several respects. The use of this nomenclature arose many years ago from psychophysical studies of human subjects who displayed reduced reactions to painful stimuli applied to skin areas pretreated with capsaicin [36]. Unfortunately, once the
capsaicin receptor (TRPV1) was recognized as a unique molecule entity, the psychophysical use of ‘desensitization’ evolved into pharmacological use, which denotes the reduction of responsiveness of an ion channel-receptor complex following prolonged or repeated agonist exposures. In the continued presence of exogenous agonists such as capsaicin, pharmacological desensitization of TRPV1 itself may indeed contribute acutely to analgesic efficacy. However, transient effects on TRPV1 are quite unlikely to account for the persistent pain relief seen clinically following either single treatments with high-concentration capsaicin or repetitive administration of low-concentration capsaicin. Hence, in my opinion, the least inaccurate term for the persistent local effects of capsaicin is ‘defunctionalization’, which avoids conceptual confusion with the intrinsic desensitization of the TRPV1 receptor. The multiple aspects of capsaicin-induced loss of sensory nerve function have been appreciated for many years [23]. Degeneration or necrosis may occur, but the absence of a panel of antigens is required to determine that nerve terminals are truly absent.

DOSE RESPONSE AND THE MITIGATION OF PUNGENCY

Evolutionary selective pressures seem to have maximized the pungency of capsaicin. According to the ‘directed-deterrence’ hypothesis, the key driver for the expression of capsaicin may be that the compound’s pungency is able to deter ambulatory animals from eating chili pepper fruits, with selective pressures favoring those plants whose seeds were dispersed widely by birds [37]. The TRPV1 receptor of birds is not activated by capsaicin [38], and hence they are undeterred from ingesting chili pepper fruits and can excrete the pepper seeds large distances away.

The assumption that pungency is inevitably associated with capsaicin administration has caused researchers to investigate the therapeutic potential of less pungent compounds such as resiniferatoxin [39] and olvanil [40]. From comparative studies of various TRPV1 agonists, it appears that the rate of channel activation is the best predictor for pungency, with capsaicin being the most pungent and most rapidly activating compound [41].

Long ago it was hypothesized that if capsaicin were delivered to a target sensory neuron sufficiently quickly that the defunctionalization process could occur almost as rapidly as the activation process, thereby rendering well tolerated the capsaicin-based treatment [42]. Clinical data to support a capsaicin exposure-pungency relationship which plateaus has been provided by the clinical development program for Qutenza and its successor formulation. Qutenza was well tolerated in clinical studies, with greater than 98% of subjects being able to complete 30- to 60-minute treatment durations without asking for early removal of the patches [43]. These results might be surprising if the pain associated with the treatment procedure of a formulation containing 80-fold more capsaicin than the highest concentration OTC cream were expected to be 80 times more pungent. Even more instructive is the clinical data for NGX-1998, which is the successor non-patch/liquid formulation of capsaicin. A Phase 2 study of NGX-1998 in patients with postherpetic neuralgia revealed excellent tolerability to the treatment procedure even in the absence of a local anesthetic pretreatment [11, 44]. There were no differences in tolerability between the 10% and 20% w/w versions of NGX-1998 even though the 20% form displayed more efficacy. It is likely that future clinical studies will utilize only 20% capsaicin.

Combined with preexisting theory, data emerging from the development programs of the high-concentration topical formulations Qutenza and NGX-1998 enables inferences about the dose-response relationship of topical capsaicin.
Two types of outcomes must be considered: persistent pain relief and short-term pungency and tolerability. It must also be recognized that a topical dose is constituted not only by the formulation’s drug concentration but also the duration of application, plus the volume for liquid formulations such as NGX-1998. Overall, the shape of the capsaicin-pain relief dose-response curve is rather shallow, with only modest benefit generally observed with 60- or 90-minute patch applications of Qutenza relative to 30 minutes. One articulation for these data is that for each single hyperactive cutaneous nociceptive nerve fiber, there is a ‘tipping point’ at which a capsaicin exposure results in irreversible damage and degeneration. Bringing a sufficient number of hyperactive cutaneous nociceptors in a painful area to their tipping point and causing their defunctionalization/degeneration can yield discernible pain relief.

With respect to short-term tolerability, the dose-response curve appears to become flat at high concentrations and may become bell-shaped. As is depicted in Fig. (4), high-concentration topical capsaicin formulations may push nociceptors into a defunctionalized state almost as quickly as they are activated, thereby reducing sensations of pungency and thereby improving tolerability.

**IMPORTANCE OF LOCAL CONCENTRATIONS, DELIVERY RATE AND FORMULATION COMPONENTS**

The skin pharmacokinetics of drug substances and formulation excipients released by topical formulations are sometime either unstudied or unreported. All of these data are important for consideration of potential secondary pharmacokinetics of applied drug substances and evaluation of possible actions of ‘inactive’ formulation components.

Cutaneous drug delivery is very inefficient – meaning that often only 1 to 3% of the applied drug will partition into skin – so excessive amounts are applied to increase the concentration gradient. Adding complexity is that drugs substances may be preferentially localized or solubilized in just one component of the formulation. For instance, the overall concentration of capsaicin in the Qutenza patch is 8% on a percentage w/w basis. However, capsaicin is mostly contained within the patch in microreservoirs in which it is solubilized at 28% w/v in a skin penetration enhancer. To consider the implications of this concentration, 28% is roughly equivalent to 918 mM. Similarly, the approximate concentration of capsaicin in the 20% NGX-1998 formulation is 656 mM. Assuming 2% or less of the capsaicin is absorbed into the skin, it is not possible to arrive at a prediction of concentrations in the epidermis or dermis due to the significant deportion which will occur in the stratum corneum [45]. The potency of capsaicin at TRPV1 at 32 °C is less than 0.1 nM and (as was discussed above) secondary pharmacodynamic effects may begin in the range of about 3 µM. It remains unknown to what degree topically applied capsaicin can attain the latter concentration.

To illustrate the complexities of skin pharmacokinetics, consider an innovative model to study skin reinnervation after complete denervation developed at Johns Hopkins University [46]. The model is based upon a 48-hour application of a 0.1% w/w capsaicin cream under occlusion to the distal legs of either healthy control or patients with peripheral neuropathies. The capsaicin cream is removed and reapplied after 24 hours. Immunohistochemical analyses of punch biopsies reveal that essentially all intra-epidermal nerve fibers are lost. Another group has reported that in addition to nearly complete loss of PGP 9.5 immunostaining, when applied to the volar forearm, reductions in sudomotor, vasomotor, pilomotor and sensory functions and the densities of associated nerve fibers occurred [47]. The authors of this study find the loss of not only sensory but also sympathetic and parasympathetic nerve fibers worrisome and concluded that, “…caution should be taken when topical capsaicin is applied to skin surfaces at risk for ulceration, particularly in neuropathic conditions characterized by sensory and autonomic impairment.”
The observation of ablation of nerve fibers not restricted to a subset of sensory fibers in the publication above contradicts numerous previous studies with capsaicin. Moreover, given the substantial and rapid delivery of capsaicin from Qutenza, it is somewhat surprising that the high-concentration patch reduces IENF density less than other treatments: based on PGP 9.5 analyses, with the Johns Hopkins model and rigorous repeated applications of over-the-counter (OTC) low-concentration creams [20] essentially all IENFs disappear, whereas with Qutenza only 59 to 80% of IENFs are lost [21, 48]. The complete disappearance of IENF immunostaining could be explained if the 48-hour occlusive administration of the Johns Hopkins model or repeated applications of OTC creams resulted in an accumulation of more capsaicin in the skin than is provided by Qutenza. However, in a robust porcine model of capsaicin skin pharmacokinetics, application of the same 0.1% cream for 48 hours under occlusion did not induce a quantifiable difference in capsaicin content relative to a 1-hour administration of a 0.075% cream, and both of these treatments provided substantially less skin capsaicin than followed a single Qutenza application for 60 minutes (S. Babbar, personal communication).

For an alternative explanation of the effect size on IENF density, it may be interesting to consider the excipients of the topical creams. The Johns Hopkins model has consistently employed Capzasin® (0.1%) HP Arthritis Pain Relief from Chattem, Inc. (Chattanooga, TN, USA). The following are the inactive ingredients, which are listed in descending order of predominance: benzyl alcohol, cetyl alcohol, glyceryl stearate, isopropyl myristate, PEG-40 stearate, petrolatum, sorbitol and water. There is no concentration information required for ‘inactive’ ingredients in OTC products. Whatever the concentration, benzyl alcohol is certainly of interest, as topical administration appears to lead its accumulation in the epidermis [49] and benzyl alcohol has long been recognized to be a neurotoxin [50, 51]. Detailed analyses of the underlying mechanisms of neurotoxicity have recently shown that benzyl alcohol is a mitochondrial toxicant capable of compromising mitochondrial integrity and initiating pro-apoptotic cascades [52]. Unfortunately, there is no information about whether the control cream employed by groups using the Johns Hopkins model contains any benzyl alcohol. Repeating the above experiments with a true matching control cream may be necessary, and it would be extremely interesting to apply benzyl alcohol under occlusion for 48 hours and determine effects on nociceptors. It is also possible that benzyl alcohol has an additive or even synergistic neurotoxicity with capsaicin, which might explain the loss of all types of nerves fibers observed by Gibbon and colleagues [47]. As a general rule, before any rigorous efficacy or safety inferences can be made regarding a drug substance, it is necessary to exclude the potential effects of excipients which may not be inactive at the high concentrations attained in the skin.

SECONDARY PHARMACODYNAMICS AND POTENTIAL COMMON MECHANISMS WITH TOPICAL LIDOCAINE

Lidocaine is also widely used as a topical analgesic. In particular, the Lidoderm® (5% w/w lidocaine) patch was approved in the US in 1999 for the management of postherpetic neuralgia. The same patch is sold in the EU and other countries under the trade name of Versatis® [53]. Lidoderm patches are applied directly to the painful area and worn chronically for 12-hour cycles. Unlike topical formulations intended to produce full anesthesia, the generally attributed analgesic mechanism of action of Lidoderm is that the sub-anesthetic concentrations of lidocaine provided by the patch selectively inhibit the activity of hyperactive cutaneous nociceptors [54]. Interestingly, a combination QST-biopsy study reveals that Lidoderm not only elevated thresholds for nociceptor activation at the application site, but also decreased IENF densities after 41 days of administration (see Fig. 5A) [55].

How is the topical lidocaine reducing IENF density? In contrast to the high selectivity of capsaicin for the TRPV1 receptor at concentrations less than 1 μM, lidocaine appears to have multiple effects at concentrations that are considered therapeutically relevant for inhibition of voltage-activated sodium channels. In the case of Lidoderm, which is a 5% w/w patch in an aqueous matrix, the overall concentration of lidocaine in the patch is calculated to be 213 mM. Three percent cutaneous bioavailability has been reported for Lidoderm [56], but this value reflects both cutaneous and transdermal delivery. In a study of human with a 5% cream...
formulation, the delivery to skin was 109 ng/mm² [57], but no concentration information was provided on a molar basis. Like capsaicin, lidocaine will depot in the stratum corneum, but because it is less lipophilic than capsaicin, the degree of depoting should be less [45].

In addition to the best known activity as a use-dependent Na⁺ channel blocker, lidocaine and its main metabolite inhibit hyperpolarization-activated and cyclic nucleotide-gated (HCN) channels that contribute to pacemaker currents [58]. Lidocaine can also directly activate TRPV1 and modulate activation by capsaicin [59]. Importantly for the present discussion, millimolar lidocaine concentrations are known to be neurotoxic. In cultured rat sensory neurons, lidocaine induces morphological changes such as varicosity formation and apoptosis [60]. Direct effects on mitochondria may underlie the neurotoxicity (see Fig. 5B) [61, 62], possibly suggesting a shared mechanism with capsaicin.

The importance of delivery rate and excipients call into question the notion that ‘bioequivalence’ can be demonstrated for a topical analgesic based solely on cutaneous drug concentrations. Total accumulation of a drug in the skin may not fully reflect or predict efficacy or adverse effects: drug concentration, duration of application, number of times applied per day, rate of delivery, whether applied under occlusion and excipients all matter. Recently, Endo Pharmaceuticals filed a Citizen’s Petition to the FDA regarding generic versions of Lidoderm which raised many of these same issues [63]. Endo argues that skin pharmacokinetic studies are not sufficient and that clinical efficacy studies must be conducted to prove that two topical products produce the same degree of pain relief.

CONCLUSIONS

Even though topical capsaicin has been used for centuries as an analgesic, the first formal review of capsaicin by drug regulatory agencies occurred during the recent approval process for a single-use high-concentration topical capsaicin patch to manage peripheral neuropathic pain. The clinical validation of Qutenza and the continued success of Lidoderm/Versatis have reinvigorated interest in topical approaches to pain management.

Changes in cutaneous innervation result from many pain syndromes and other disorders. As a general rule, the severity of innervation loss seems to positively correlate with degree of pain. This relationship underlies the often noted paradoxical nature of peripheral neuropathies: pain intermingled with numbness. Functional evaluations of cutaneous innervation are very useful, but for the purposes of comparing across studies or institutions, data from immunostaining of punch biopsies with antigens such as PGP 9.5 can more readily be discussed in review articles such as this one. However, it should never be assumed that PGP 9.5 immunostaining can tell the whole story, particularly for regenerating cutaneous nerve endings.

Clear reductions of PGP 9.5 immunostaining occur following topical administration of capsaicin. Such reductions are not restricted to the epidermis but also occur in the dermis. However, because of the complexity in quantifying reductions in the dermis, these values are usually not determined. Little or no information is available on the depth of capsaicin penetration and to what degree it ablates nociceptive fibers in the dermis.

It is wrong to focus exclusively on the concentration of drug content when evaluating or comparing topical formulations: concentration, duration of application, applications per day and excipients all matter. Skin concentrations of drug substances and excipients may be quite high and induce both primary and secondary pharmacodynamic responses. Excipients that are normally benign or inactive when diluted in large volumes may accumulate in skin layer and produce toxicities. Mitochondria-rich cutaneous nociceptors are perhaps the most vulnerable structure in the skin, and mitochondrial toxicants may readily cause collapse of the highly metabolically active nociceptive nerve terminals. Topical capsaicin and lidocaine share an ability to cause the localized degeneration of nociceptors, possibly through deleterious effects on mitochondria.

Another implication of the complexity of topical formulations is that efficacy or safety of drug produces such as Qutenza cannot be based upon data from OTC products or experimental models. In particular, data from the Johns Hopkins models showing the loss of all cutaneous nerve fibers should not be considered relevant for predicting potential adverse effects of Qutenza or other topical capsaicin formulations. ‘Bioequivalence’ based upon capsaicin delivery to skin is not a helpful concept – each formulation requires clinical data to confirm that it actually produces pain relief, irrespective of how much of a drug substance is delivered into skin.

In summary, even though the skin is the most accessible of all human organs, much remains to be learned about how its innervation changes and the effects of drugs on that innervation. The pharmacokinetics and pharmacodynamics of topical analgesics such as capsaicin and lidocaine should not be assumed to be less complex than orally administered analgesics.

CONFLICT OF INTEREST

Keith Bley was formerly employed by NeurogesX, Inc., which developed and marketed the Qutenza (8% capsaicin) patch.

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