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Clinical Significance of Topical Spermidine Hyaluronate in Vestibulodynia: An Early Appraisal

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Abstract

Background: The most common subtype of chronic vulvar pain is provoked vestibulodynia. The entry of the vagina is the site of acute and recurrent pain in this highly prevalent and debilitating condition, which is characterized by pressure application or attempted vaginal penetration. The aim of this study was to determine the effectiveness of topical spermidine in patients with vestibulodynia. **Methods:** Topical gels containing spermidine in hyaluronate complexes Ubi1 and Ubi2 endowed with differentiated release ratio and viscosity were applied at 3 doses/week during 4-weeks, then at 2 doses/week during the next 4-weeks in two groups of patients. Pain relief was measured by visual analogic score (VAS) and dyspareunia score expressed as percent improvement from baseline to posttreatment. **Results:** Group 1 treated with Ubi1 provided improvement in pain (46%) and dyspareunia (27%). However, the treatment in Group 2 resulted in a superior amelioration: VAS of pain (76%) and dyspareunia (50%) as Ubi2 gel provided higher dose and viscosity along with improved local application. **Conclusions:** Our results demonstrated that preparation 2 resulted in greater reduction in symptoms as compared to preparation 1 as measured by the VAS and Marinoff scale. These early, yet outstanding clinical outcomes in vestibulodynia through to the stimulation of tissue mechanosensor and their relevant downstream effects are reviewed hereafter.

Keywords

Vestibulodynia, Spermidine, Vulvodynia, Dyspareunia, Vulvar Pain.

1. Introduction

Vulvodynia is a persistent pain disorder, which lasts at least 3 months [1] [2].

It was estimated that between 10% and 18% women suffer from vulvodynia during their lifetime [3]. Psychological well-being of these women is adversely affected by relevant impairment of their sexuality, relationships, and self-image. In the meantime, vulvodynia requires relevant costs related to difficulty to obtain a diagnosis, needs of consulting many gynecologists to obtain a diagnosis, and many women suffer for years before receiving a proper treatment [4]. The most common subtype of vulvodynia (about 80%) is provoked vestibulodynia (VBD), in which the pain is localized to the vulvar vestibule, and it is triggered by touch and/or upon an attempt of vaginal penetration (eg, intercourse or tampon use) [1] [2]. The pain of VBD may be described as burning, discomfort or excruciating pain and is often accompanied by dyspareunia. In many cases, patients with VBD cannot tolerate anything touching their vulva, such as underwear and tight pants, or sitting for prolonged time, all of which may trigger pain. The pathogenesis of vulvodynia is poorly understood, and the etiology is multifactorial. Several factors are suggested to involve in the development of VBD. Multiple pathophysiological factors are suggested to involve in the development and persistence of VBD [5] [6], and various mechanisms could contribute at different times during disease.

A multifactorial etiology, such as recurrent candidiasis and vulvovaginal infections, hormone alterations, inflammation, allergy, genetic predisposition, and psychogenic vulnerability that promote vestibular nerve fibers sensitization have been implicated in the development and maintenance of VBD [6]. In addition, the discomfort inherent in VBD is often associated with muscular hyperactivity of the pelvic floor [7]. Hypersensitivity of the vulvar vestibule is a defining characteristic of VBD [7]. The pattern of VBD responses is suggestive of sensory abnormalities in the form of evoked pain, suggesting sensitization, an underlying manifestation of neuropathic pain. This is consistent with biopsies that showed increased innervation of the vulvar vestibule, subepithelial heparinase activity, and cytokines, which have been linked to a neuroinflammatory process [4].

It is becoming increasingly apparent that VBD is likely not a disease, but several diseases, in which the end point of various factors in which the end point of different factors are the vestibular hypersensitivity and the pelvic floor hypertonic dysfunctions. VBD can be considered a summation and overlapping of different trigger factors with "weight" and "predominance" different in each single patient. The identification of the main associated factors in each woman has significant treatment implications, and highlights that there is no single approach to treatment. Different therapies have been proposed over time to treat the disease (pharmacological options, pelvic floor physical therapy, hormonal agents, topical actives and psychological interventions), but there is no general consensus, and recommended guidelines are mainly based on expert opinion, case series and the limited number of placebo controlled randomized clinical trials are only for the treatment of VBD [8]. Spermidine is an arginine derivative with an essential role in all living cells, playing a key role as posttranslational

cellular modulator via electrostatic interactions including chromatin compaction [9], cytoskeleton build-up through microtubule elongation [10] [11], and the phenotype renovation linked to the promotion of autophagy [12]. Another proposed function for spermidine is the role in protecting cells from reactive oxygen species damage (ROS) [13]. It was demonstrated spermidine's trophic modulatory action on female tissues when released from supramolecular complexes (SMC) [14]. The polycationic spermidine was found to express its optimal potency in regeneration if sustained released by SMC formed with the hydrocolloid hyaluronan (HA), an elected as anionic counter-pair for its synergistic trophic activity coupled with high biocompatibility. This study sought to determine the efficacy of spermidine in VBD and to verify the SMC release profile at two and spermidine concentrations as well as the impact of viscosity related to the HA's molecular weight in terms of clinical performance.

2. Materials and Method

2.1. Study Population and Trial Design

The study population included patients with VBD who met the follow inclusion criteria: women at least 18 years of age and before the menopause (absence of menstruation for 12 months); experience of moderate to severe pain (minimum of 5/10 on a numerical rating scale in at least 90 % of attempted sexual intercourse); pain limited to the vestibule during vaginal intercourse and during activities exerting pressure on the vestibule (tampon insertion, tight jeans or pants, cycling, horseback riding), and presence of VBD for at least 6 months and diagnosed according to the standardized gynecological examination protocol by one of our staff gynecologists. At enrollment the patients underwent gynecological evaluation to exclude any vulvovaginal disease and a vestibular cotton touch (swab test) was performed, quantified by a 0-3 score (0 = negative; 1 = weak positive; 2 = positive; 3 = strong positive).

2.2. Treatment

Patients who consented to enroll in the study and after signed informed consent were randomized to one of two arms, which consisted of Spermidine in gel at two concentrations released from SMC made with low and high molecular weight hyaluronic acid (LMW-HA and HMW-HA, respectively). The gel (Ubi1) used for Group 1 was low viscosity (≈5000 cps) 3% SMC, spermidine-LMW-HA (MW = c.a. 200 kD; Resilen™; supplier: Kyowa-Hakko, Japan) at spermidine/HA ratio = 1:75 meq/meq. The gel used for Group 2 (Ubi2) was high viscosity (≈5000 cps) 2% SMC, spermidine-HMW-HA (MW = 1.5 - 2.2 mD; supplier: Esperis, Milan, Italy) at higher spermidine/HA ratio = c.a. 20 meq/meq. Dosage forms packaged in 30-mL airless tubes registered as medical devices (Tixupharma, Milan) were produced on a UNI EN ISO 9001 and ISO 13485 certified premises. Randomization was generated by a computer program with a 1:1 allocation.

Subjects were instructed to apply a fingertip amount of gel (≈2 ml) onto the

vestibular area 3 times weekly during 4-weeks, then twice-a-week for the next 4-weeks.

The investigational products, namely Ubi1 and Ub2, have been delivered to the clinical site in identical of packaging and appearance, except for a higher viscosity of the former compared to the latter. This feature was undisclosed to both the Investigator and individual patient—who did not have a comparative view of the alternative product—therefore a double-blind randomization could be carried out.

2.3. Outcome Measures

Primary clinical endpoint was the difference in the individual patient's vestibular burning/pain measured on a discrete visual analog scale (VAS) of 0 (no pain) to 10 (worst possible pain). Dyspareunia was also evaluated with the Marinoff scale: Level 0 or no pain; Level I or causes discomfort but does not prevent sexual intercourse; Level II or frequently prevents sexual intercourse; Level III or completely prevents sexual intercourse.

2.4. Statistical Analysis

The evaluation of the difference between pretreatment (T0) and posttreatment (T1) after eight weeks of therapy for both Ubi1 and Ubi2 containing spermidine complexes was performed using the Wilcoxon signed rank test for paired data. The differences from baseline and posttreatment were evaluated using the Mann-Whitney rank sum test for independent data. The level of significance was 0.05.

3. Results

A total of 26 patients consented to participate in the study. All women recruited, 13 in each group, completed the trial. The two groups all of Caucasian origin, did not significantly differ in terms of age ($p = 0.838$), gravity of the disease status assessed by cotton swab test ($p = 0.597$), and symptoms as set forth in **Table 1**.

In Group 1, a vestibular pain decreased from 7.3 ± 0.49 to 3.9 ± 2.48 ($p < 0.001$), while dyspareunia reduced from 2.8 ± 0.43 to 2.1 ± 0.94 ($p = 0.004$) (**Table 2**). Group 2 showed greater reduction in both vestibular pain, decreased from 7.1 ± 0.83 to 1.8 ± 1.04 ($p = 0.008$) and dyspareunia, went from 3.0 ± 0 to 1.5 ± 0.76 ($p = 0.016$) (**Table 2**). These differences were statistically significant. Outcomes of Groups 1 vs 2 differ profoundly both in quantitative and qualitative terms, as illustrated by the box plot of **Figure 1**. The Ubi1 treated Group 1 experienced a mild improvement of VAS pain score, moreover with scattered distribution. The differences in response in the two Groups were statistically significant for both VAS ($p = 0.038$) and dyspareunia ($p = 0.039$). A broader dispersion of VAS in Group 1 vs Group 2 confirms that a high gel consistency is mandatory. No adverse effects occurred in any group during the study period.

Table 1. Baseline characteristics of the two study groups

	Group 1 (n = 13)	Group 2 (n = 13)	P value
Age	33.3 (10.9)	32.4 (11.0)	NS
Cotton swab test	2.8 (0.4)	3.0 (0.1)	NS
Burning/pain (VAS)	7.3 (0.8)	7.1 (0.8)	NS
Dyspareunia score*	2.8 (0.4)	5.5 (0.8)	NS

Data are expressed as mean (SD). *Marinoff scale. Abbreviation: Visual analogue scale (VAS); NS, not significant.

Table 2. Change of symptoms and signs at baseline and after therapy.

	Group 1 (n = 13)				Group 2 (n = 13)			
	Baseline	Post-therapy	% improvement	p value	Baseline	Post-therapy	% improvement	p value
Burning/pain (VAS)	7.3 (0.8)	3.9 (2.4)	46%	0.001	7.1 (0.8)	1.8 (1.0)	76%	0.008
Dyspareunia score*	2.8 (0.4)	2.1 (0.9)	27%	0.004	3.0 (0.1)	1.5 (0.7)	50%	0.016

Data are expressed as mean (SD). *Marinoff scale. Abbreviation: Visual analogue scale (VAS); NS, not significant.

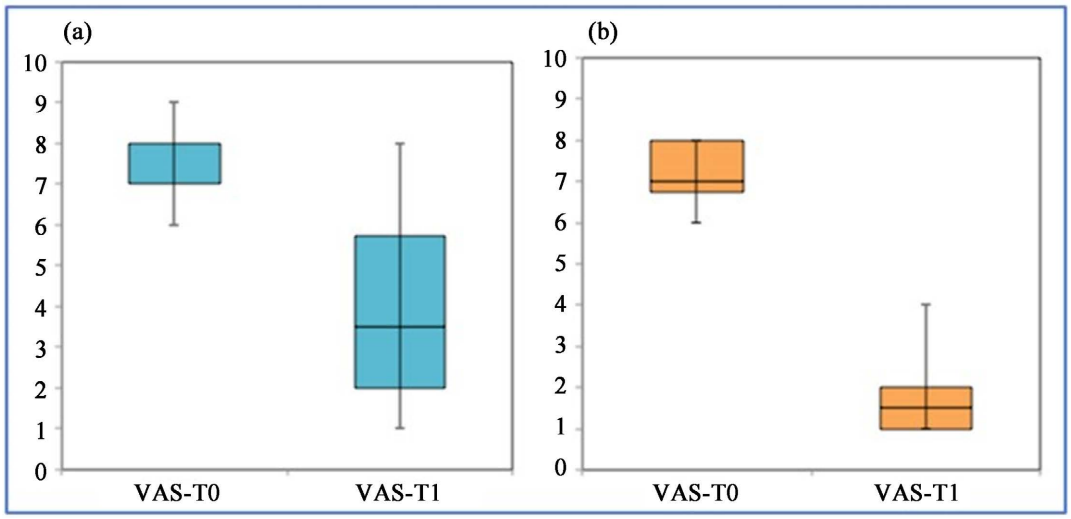

Figure 1. Box plot of VAS pain scores among women at baseline and posttreatment in Group 1 (a) and in Group 2 (b).

4. Discussion

Our study showed that topical spermidine can be effective in reducing pain and improving sexual function in VBD. Neuro inflammation and vestibular neuroproliferation with an increased density of C-afferent nociceptors, are the two main mechanisms that might lead to the development of VBD [15] [16]. Noxious insults trigger inflammatory mediators, neurotransmitters, and growth factor activity, which can result in nociceptor sensitization or ectopic excitability of afferent neurons. Studying vulvodynia immune histology, several groups report

mast cell predominant inflammation [16]. Their activation is associated with the discharge of various mediators from the granules, such as NGF, tryptase, and bradykinin. Moreover, elevated concentrations of IL-1 beta and TNF-alpha have been found in women with VBD relative to asymptomatic controls [17]. Hypotrophic vestibular features can be highlighted in many patients with VBD [18]. A lower vaginal level of steroidogenic metabolites found in women with PVD, could lead to alterations in mucosal structure and thinning of the mucosa of the vestibule rendering the tissue more vulnerable to infection, inflammation, atopy and microtrauma [19]. Our positive results related to spermidine use in VBD may be attributed to its regenerative action. A multifarious regenerative process prompted by spermidine on endothelial, epithelial, and peripheral neuronal fibers seems to work on treated subjects (**Figure 2**). The overall reinforcement of the epithelium is favored by the activation of mechano-sensing elements and antioxidant/autophagy promoters leading to the spermidine-mediated activation of the epithelium, endothelium, and peripheral nerve barriers, which moderate the release of inflammatory mediators. Multiple consequences include the interrupted supply of immature mast cells; the downregulation of N-cadherin required for mast cell degranulation; the inhibited release of tumor necrosis factor (TNF)-α, interleukin (IL)-1, and matrix metalloproteases (MMP)-24 and -9, that would otherwise facilitate the recruitment of mast cell and the macrophagic phenotype activation. The "improved barrier" theory has found confirmation in studies on intestinal enterocytes: the stabilization of the epithelial layer occurs through improvements in adherent and tight junctions via c-myc transcription factors of occludin, E-cadherin and zonula occludens (ZO)-1,2 occur under the tight control of spermidine and other polyamines [20]. Another relevant action of spermidine stems from its antioxidant capacity, a protective effect evidenced by the reduction of oxidative and inflammatory markers. In macrophage and zebrafish models, spermidine moderates the accumulation of reactive oxygen species (ROS) and inhibits proinflammatory mediators such as nitric oxide (NO), prostaglandin E2, and cytokines including TNF-α and IL-1β without significant cytotoxicity [21]. The antioxidant activity of spermidine is exerted through the rescue of autophagy, as specifically observed on the female reproductive organs [22]. Noteworthy, spermidine and spermine are pivotal in the reproductive physiology, being key metabolites in both female and male reproduction steps including fertilization and embryo/fetal development. As part of essential regulator machinery, converse spermidine paucity may lead to infertility and arrest in embryogenesis. Spermidine and spermine also play a key role in embryo implantation, decidualization, placental formation and function, while the privation during gestation results in intrauterine growth retardation. In male re-production, spermidine concentration correlates with stages of spermatogenesis, and promotes sperm motility [23].

The spermidine-hyaluronate complex primary works via microenvironmental activation of the key cellular mechanosensors on the focal adhesion machinery,

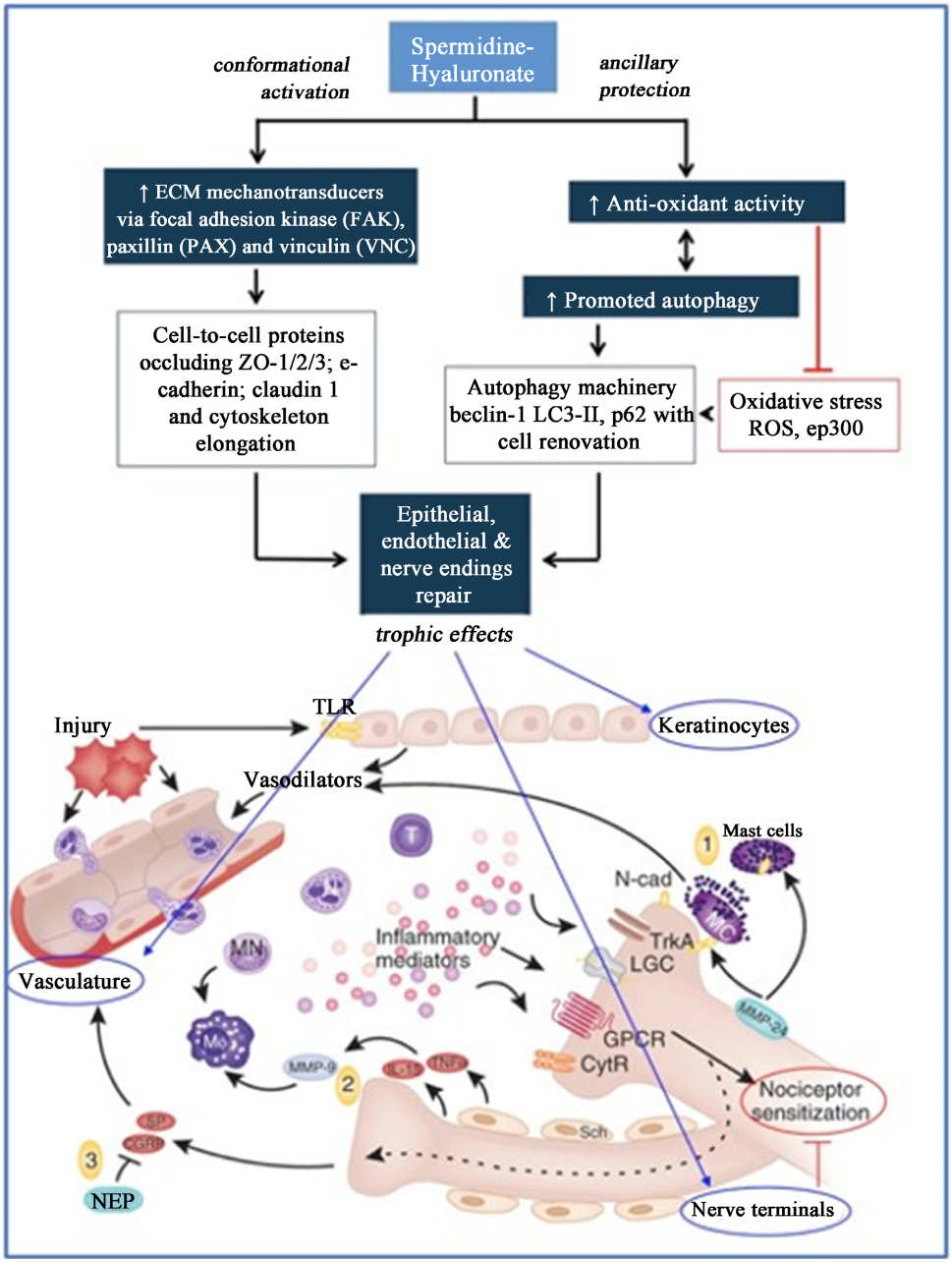

Figure 2. B Schematic view of the assumed mode of spermidine action in vulvodynia. Abbreviations: Toll-like receptors (TLRs); mast cells (MC); T cells (T); neutrophils (N); monocytes (MN); cyto-kine receptors (CytR); G protein–coupled receptor (GPCR); ligand-gated channels (LGC); tyrosine kinase re-ceptor type 1 (TrkA); substance P (SP); tumor necrosis factor (TNF)-*α*; interleukin (IL)-1; matrix metalloproteasis (MMP).

which in turns evokes biomechanical cues comparable to a 'soft' tissue rehabilitation, then leading to the progressive vulvovaginal regeneration. The adhesion receptors located on the external cell membrane bind to surface molecules (ECM) components or other cells. The signal is converted to force-induced conformations within mechanotransductor consisting in a network of cytoskeletal filaments that transmit the external forces. The strength of the initial mechano-

sensing event sensed at the cell membrane stimulates a feedback system within the linked cytoskeletal network. Following this response, cells activate contractile filaments, proteins linking the ECM to the cytoskeleton and membrane. The mechanosensitive sites along these filaments promote a second wave of responses: force-induced membrane tension, compliance or curvature leading to focal adhesion assembly [24] [25], cytoskeleton re-distribution [26] [27], cell movement [5], and ECM remodeling [28]. Mechanosensing can be active or passive. Passive mechanosensing is also known as "outside-in" mechanosensing, as it is defined by external forces being detected and transduced [29]. These forces include tension, compression, shear stress and hydrostatic pressure. Conversely, active mechanosensing is known as "inside-out" mechanosensing and is defined by internal forces being generated by the cell to detect changes in the external environment: cellular traction or electrostatic cue that mimics external cues, as those generated by spermidine-hyaluronate complex. The switch to Ubi2 was needed to improve the moderate outcomes from the first panel, that is, to pursue of a higher release ratio coupled with enhanced viscosity and muco-adhesive enabled by HMW-HA. Such differences seem attributable to the poor consistency of the Ubi1 gel. We can hypothesize that a scarce adhesion did not give sufficient contact time for the local release of spermidine, as the reduction of vulvar pain and dyspareunia were 46% and 27%, observed between the two study groups. In fact, sub-jects in Group 2 testified a better applicability of the high-dosed gel whose enhanced viscosity is coupled with a short drying time (3 to 4 minutes). The optimized formulation allowed spermidine to act providing a reduction of vulvar and sexual pain of 76% and 50%, respectively. The prolonged contact time seems to support a sustained release modality, thus providing a regular spermidine gradient, which better implements its remodeling activity. The lack of a placebo and control group, the limited sample, and follow-up without treatment are some of the potential limitations of our study. How-ever, data collected during our trial demonstrated that patients with VBD treated with spermidine safely experienced significant, consistent, and clinically meaningful improvements in pain and dyspareunia. This investigation highlights the importance of exploring this new approach to vulvodynia management, still in need of more effective therapies. Additional research might be helpful to define new frontiers to mitigate the sufferers' conditions and further frame the complexity of persistent vulvar pain. Possible future directions might include real-world data gathering regarding the usage of spermidine in routine clinical practice as a multimodal therapy in a larger vulvodynia population.

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after the study termination.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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