Topical cholesterol/lovastatin for the treatment of porokeratosis: a pathogenesis-directed therapy

Lihi Atzmony, MD, Young H. Lim, BS, Claire Hamilton, MD, PhD, Jonathan S. Leventhal, MD, Annette Wagner, MD, Amy S. Paller, MD, Keith A. Choate, MD, PhD

PII: S0190-9622(19)32648-9
DOI: https://doi.org/10.1016/j.jaad.2019.08.043
Reference: YMJD 13764

To appear in: Journal of the American Academy of Dermatology

Received Date: 10 June 2019
Revised Date: 13 August 2019
Accepted Date: 20 August 2019


This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2019 Published by Elsevier on behalf of the American Academy of Dermatology, Inc.
Article type: Original article

Title: Topical cholesterol/lovastatin for the treatment of porokeratosis: a pathogenesis-directed therapy

Lihi Atzmony, MD1,2,3, Young H. Lim, BS1,2,4, Claire Hamilton, MD, PhD1, Jonathan S. Leventhal, MD1, Annette Wagner, MD5, Amy S. Paller, MD5, Keith A. Choate, MD, PhD1,2,4

1Department of Dermatology, Yale University School of Medicine, New Haven, Connecticut, USA
2Department of Genetics, Yale University School of Medicine, New Haven, Connecticut, USA
3Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel
4Department of Pathology, Yale University School of Medicine, New Haven, Connecticut, USA
5Departments of Dermatology and Pediatrics, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA.

Corresponding author: Keith A. Choate MD, PhD
Departments of Dermatology, Genetics, and Pathology
Yale University School of Medicine
333 Cedar St, New Haven, CT 06520 (Keith.choate@yale.edu)

Funding sources: This study was supported in part by the National Institutes of Health (R01 AR071491) to Dr. Choate and the Yale Center for Mendelian Genomics (U54 HG006504). Dr. Atzmony was supported by Davidoff Foundation.

Conflicts of Interest: None declared.
IRB approval status: The genetic study was approved by the Yale Human Investigation Committee; approval #0809004252.

Reprint requests: Keith A. Choate

Manuscript word count: 2,359 words

Abstract word count: 199

Capsule summary word count: 47

References: 33

Figures: 4

Tables: 1

Keywords: porokeratosis; disseminated superficial actinic porokeratosis; linear porokeratosis; therapy; topical therapy; genetic skin diseases, genetics; medical dermatology; pediatric dermatology; statins; cholesterol; mevalonate pathway.
ABSTRACT

Background: Porokeratosis is associated with mevalonate pathway gene mutations. Therapeutic options are few and often limited in efficacy.

Objective: On the basis of preventing the accumulation of toxic metabolites while replenishing essential end-products, we studied the efficacy of topical lovastatin/cholesterol in different variants of porokeratosis.

Methods: A series of 5 patients with disseminated superficial actinic porokeratosis (DSAP, n=1), porokeratosis palmaris et plantaris disseminata (PPPD, n=2) and linear porokeratosis (LP, n=2) were enrolled. Patients were genotyped prior to initiation of therapy and then applied topical lovastatin/cholesterol twice daily to a unilateral defined treatment area for up to 3 months. Response was evaluated and patients were photographed every visit.

Results: Three patients had MVD mutations and 2 patients had PMVK mutations. Treatment with topical lovastatin/cholesterol (but not cholesterol alone) resulted in near complete clearance of DSAP lesions after 4 weeks of therapy, and moderate improvement of lesions in PPPD and LP. There were no adverse events.

Limitations: Case series design with a small number of patients.

Conclusion: Topical cholesterol/lovastatin is a safe and effective therapy for porokeratosis that underscores the utility of a pathogenesis-based therapies which replace deficient end-products and prevent accumulation of potentially toxic precursors.
INTRODUCTION

Porokeratosis is a heterogenous group of keratinization disorders subclassified based on clinical appearance. Variants include disseminated superficial actinic porokeratosis (DSAP), disseminated superficial porokeratosis (DSP), porokeratosis of Mibelli, porokeratosis palmaris et plantaris disseminate (PPP), and linear porokeratosis (LP). All variants share the histopathological feature of a cornoid lamella, a vertical column of parakeratosis situated above dyskeratotic cells within the granular layer. Familial cases with an autosomal dominant mode of inheritance, as well as sporadic cases, have been described. DSAP is the most common subtype of porokeratosis, although its exact prevalence is unknown. It usually affects individuals in their 30s and 40s with a slight female predominance. Lesions appear on sun-exposed areas as asymptomatic or pruritic pink to brown papules or plaques with a raised railroad track border and an atrophic, sometimes hypopigmented center.

Porokeratosis is considered a premalignant condition with a malignant transformation rate of 7.5%. The most common reported malignancy is squamous cell carcinoma (SCC), but basal cell carcinomas and melanomas have also been reported. Although all subtypes of porokeratosis have an increased risk of skin cancer, linear, large, and long-standing lesions are reported to have higher risk.

As in other clonal keratinocyte disorders, treatment for porokeratosis is primarily focused on lesion destruction using cryotherapy, photodynamic therapy (PDT), CO₂ lasers, and/or 5-fluorouracil. Other strategies to reduce scale and inflammation associated with these lesions include acitretin, topical corticosteroids, and vitamin D analogs. These approaches are often ineffective and costly.

Recently, heterozygous germline mutations in the mevalonate pathway genes MVK, PMVK, MVD, and FDPS were identified in familial and sporadic porokeratosis, and second-hit somatic mutations were identified in DSAP linear porokeratosis. Together, this suggests
that individual lesions in various porokeratosis variants arise in regions affected by second-hit mutations in the genes encoding key components of the mevalonate pathway. The mevalonate pathway is essential for cell growth and differentiation, gene expression, cytoskeleton assembly and post-translational modification of proteins involved in intracellular signaling (Figure 1). Cholesterol, one of the end-products of the mevalonate pathway, is a key component of the extracellular lipid matrix in the stratum corneum, playing an essential role in providing and maintaining skin barrier function. Depletion of cholesterol has been reported to result in increased sensitivity of keratinocytes to stimuli driving apoptosis. Premature apoptosis and dysregulated differentiation of keratinocytes have been identified in several types of porokeratosis, supporting a simple pathogenesis model in which loss-of-function mutations in MVK, PMVK, MVD, and FDPS result in cholesterol deficiency in porokeratosis affected skin, directly leading to disease phenotype. However, as has been demonstrated in other inherited metabolic disorders, the porokeratosis phenotype may reflect both the deficiency of metabolic pathway end products and the accumulation of toxic metabolites synthesized proximally in the pathway. Genetic insights into the pathogenesis of porokeratosis provide guidance for pathogenesis- or mechanism-directed therapy that aims to correct the metabolic anomalies resulting from diminished mevalonate pathway enzyme activity. A therapeutic approach preventing the accumulation of toxic metabolites while replenishing essential end-products has been successfully utilized in CHILD syndrome, an X-linked dominant disorder of distal cholesterol metabolism. Topical application of lovastatin, an HMG-CoA inhibitor, and cholesterol led to significant improvement of skin lesions, while application of cholesterol alone (i.e., solely end product replenishment) did not correct the phenotype. Using topical rather than systemic administration of the dual regimen allows the lovastatin to bypass the first-pass effect of statin
metabolism by the liver and direct access of keratinocytes to cholesterol for efficient
transepidermal incorporation.\textsuperscript{19,20}

Given our knowledge of the contribution of mevalonate pathway dysfunction in the development
of porokeratosis, we hypothesized that applying topical lovastatin/cholesterol could alleviate
porokeratosis by both replenishing cholesterol and blocking accumulation of mevalonate
pathway toxic metabolites. We tested the application of topical lovastatin/cholesterol on patients
with PPPD, DSAP and LP.
MATERIAL AND METHODS

Participants and genetic analysis:
The genetic study was approved by the Yale Human Investigation Committee and complies with the declaration of Helsinki principles. Individual consent was obtained in writing from all participants. Genomic DNA (gDNA) was isolated via standard phenol/chloroform extraction from peripheral blood or saliva. gDNA from lesional skin was obtained from fresh full thickness skin biopsies, 1 mm cores from affected epidermis of formalin-fixed paraffin-embedded (FFPE) specimens, or cultured keratinocytes from affected skin using the DNeasy Micro Kit (Qiagen) with added deparaffinization performed for FFPE tissue. Paired analysis of whole exome sequencing (WES) of affected skin and blood/saliva were performed, as previously described.21 Mutations were confirmed with Sanger sequencing.

Treatment:
A 2% cholesterol/2% lovastatin ointment (n=4) or lotion (n=1) was applied twice a day on lesional skin with occlusion for the first 1-2 weeks depending on skin lesion thickness. Therapy continued for 6 weeks-3 months. All subjects were allowed to use emollients on untreated skin. One patient (FP100-1) applied 2% cholesterol ointment twice a day for 4 weeks on lesional skin that has not been treated with 2% cholesterol/2% lovastatin. Patients were examined at 3-4 week intervals and up to 5 weeks to 3 months for clinical response.

Assessment of clinical response: Baseline clinical photography and a biopsy from affected skin were obtained. Erythema, scaling, thickness, size and number of lesions were evaluated every visit. Photography was performed at each visit to document clinical response.
RESULTS

Clinical and histologic description of cases:

Three patients with familial porokeratosis and 2 patients with LP were included in our cohort. Patients with familial porokeratosis belonged to the same family but varied in their clinical presentation. Patient characteristics are detailed in Table 1. Patient FP100-1 had DSAP with small thin erythematous plaques surrounded by delicate keratotic edge distributed over sun-exposed aspects of the upper and lower limbs. His sister (FP100-6) and cousin (FP100-9) had a clinical presentation of PPPD with punctate papules over pressure areas of the soles and larger purple-brown thin plaques with atrophic centers and a more pronounced keratotic border distributed over the extremities. The past medical history of FP100-9 was significant for cutaneous SCC (Table 1).

LP1 was a 5-year-old girl presenting with pruritic extensive whorled-linear scaly thick pink plaques over the left side of her body since birth. LP2 was a 20-year-old male with whorls of linear pink verrucous papules and plaques on his upper extremities and left lower extremity that appeared at birth and became thicker over time. In all patients, a coronoid lamella was evident upon histopathologic evaluation. In both, there was no family history of porokeratosis.

Genetic analysis:

WES was conducted on affected and unaffected subjects from the FP kindred which identified a heterozygous MVD c.70+5G>A mutation (Table 1). The variant cosegregated with disease and was recently found by our group in a subject with LP where it was proved to affect MVD splicing. Paired analysis of blood and affected keratinocytes did not identify somatic mutations or loss of heterozygosity (Table 1). Paired WES of affected tissue and blood from LP1 and LP2 identified germline and somatic PMVK mutations (Table 1).

Response to therapy:

FP100-1, FP100-6, FP100-9, and LP2 applied a 2% lovastatin/2% cholesterol ointment twice daily while LP1 applied a 2% lovastatin/2% cholesterol lotion twice daily. All FP patients applied
the ointment on one limb (FP100-1- left upper limb, FP100-6- right shin, FP100-9- right thigh).

LP1 applied the lotion on the left side of the trunk and LP2 applied the ointment on the left upper limb.

Response to therapy in DSAP:
Decrease in scaling was noted as early as 1 week in FP100-1. After 4 weeks of therapy, there was marked decrease in erythema, scaling, and size of visible lesions (Figure 2). After this dramatic result, we sought to address the possibility that skin lesions in porokeratosis derive primarily from cholesterol depletion, but found no clinical improvement after 4 weeks of treatment with twice daily application of 2% cholesterol in the same vehicle as our compounded cholesterol/lovastatin on the right upper limb. After 3 months of combined lovastatin/cholesterol therapy, only small erythematous macules were observed in treated areas (Figure 2).

Response to therapy in PPPD:
FP100-6 was treated for 6 weeks with prominent decrease of scaling and moderate decrease of erythema (Figure 3). FP100-9 was treated for 8 weeks with prominent decrease of scaling and a moderate decrease in erythema. (Figure 3). In both patients, improvement in scaling was noticed within 4 weeks of therapy and there was no change in number and size of lesions.

Response to therapy in LP:
A remarkable decrease in scale has been noted in both patients 3-4 weeks after initiation of therapy. LP-1 was noted to have pronounced decrease in erythema and thickness after 5 weeks of therapy (Figure 4). LP-2 had decrease in thickness and scaling within 4 weeks of therapy. After 3 months of treatment he displayed a moderate decrease in thickness and residual scale over the thicker component of his linear plaque (Figure 4).

Adverse events
All patients tolerated the therapy with no adverse events. There were no reports of redness, irritation, pruritus, or allergic contact dermatitis at treatment sites. FP100-6 utilized a smaller
treatment area than other subjects due to concern for systemic absorption of lovastatin while breastfeeding.
DISCUSSION

The advent of next generation sequencing has enabled discovery of the genetic basis of skin disorders and furthered our understanding of their pathogenesis, introducing the opportunity for pathogenesis-directed treatment modalities. Here, we describe a pathogenesis-directed therapy for porokeratosis, targeting the mevalonate metabolic pathway in patients with known MVD or PMVK mutations. Most interestingly, as has been demonstrated in other metabolic disorders, replenishment of a diminished end product of the mevalonate pathway alone had no treatment effect while dual end-product replenishment and toxic metabolite inhibition with a statin resulted in varying degrees of improvement in three variants of porokeratosis. While further work is needed to definitively establish the exact mechanism of this treatment, the efficacy of the dual treatment is likely via the added inhibition of toxic metabolite accumulation.

While near complete resolution was observed in our patient with thin red DSAP plaques after topical lovastatin/cholesterol treatment, the thick or atrophic brown-purple plaques in LP and PPPD patients were noted to have a partial response after 5-8 weeks of therapy. Since the lesions of the included patients with LP and PPPD were thicker or more atrophic than the lesions of the patient with DSAP, we hypothesize that they did not achieve their maximal response and will continue to improve, as seen in CHILD syndrome using the same regimen.

Additionally, thicker lesions may achieve greater response when treated with a vehicle with greater bioavailability potential. Finally, we have considered the possibility that a deficiency in mevalonate pathway end products other than cholesterol contributes to the phenotype and additional replenishment of these products may improve response in partially responding lesions.

The exact role of topical statin application in the treatment of porokeratosis remains to be fully elucidated. Notably, our hypothesis that bioactive precursors accumulate in keratinocytes and contribute to porokeratosis pathogenesis is supported by the lack of response to exclusive cholesterol application but the exact toxic precursors and mechanism of destruction are still
Mevalonate kinase deficiency is an autoinflammatory disorder with a spectrum of manifestations, including the well-defined clinical phenotypes of hyperimmunoglobulinemia D and periodic fever syndrome (HIDS) and mevalonic aciduria. It is caused by recessive/compound heterozygous MVK mutations. While one study suggested that the inflammatory hyperresponsiveness in the disease appears to be due to lack of protein prenylation, more recent work has demonstrated that mevalonate accumulation contributes to disease pathogenesis by inducing innate immune cells via activation of IGF1-R and mTOR and subsequent histone modification in inflammatory pathways. In this study, a statin, which blocks mevalonate generation, prevented immune activation while 6-fluoromevalonate, an MVD inhibitor, augmented the induction of pro-inflammatory cytokine production, indicating that the accumulation of a molecule upstream of 6-fluoromevalonate (mevalonate) plays a role in the induction of cytokine production, excluding a role for protein prenylation. Interestingly, patients with HIDS treated with statins showed a reduced excretion of mevalonate, and a decreased number of febrile days, supporting the concept that lowering mevalonate levels is beneficial for disease activity. In contrast, patients with mevalonic aciduria, which is the more severe end of the spectrum of mevalonate kinase deficiency, flared with lovastatin treatment. Keratinocytes also play a role in the innate immunity. While this suggests that mevalonate accumulation may contribute to porokeratosis pathogenesis, and other studies of disorders of post-squalene cholesterol synthesis have shown that toxic precursor sterols play a role in disease pathogenesis, further work is needed to clarify the role of mevalonate and other potential toxic metabolites in porokeratosis pathogenesis.

We hypothesized that blockade of metabolite production alone with a statin would not be efficient and will result in adverse events based on previous murine studies that have demonstrated ichthyosiform changes with topical solvent-dispersed statin application, reflecting the key role of cholesterol in the epidermal barrier. Statin-induced cholesterol deficiency led to decreased number and internal contents of epidermal lamellar bodies, as well
as altered lamellar bilayer architecture, with no evidence of cytotoxicity.\textsuperscript{31} Decreased number of
lamellar bodies and disrupted lamellar bilayer architecture have been demonstrated in
keratinocytes beneath the cornoid lamella in porokeratosis, presumably reflecting the role of
cholesterol deficiency in porokeratosis pathogenesis.\textsuperscript{32} Since it has been previously shown that
non-physiologic (petrolatum) lipid application, which remains restricted to the stratum corneum,
produces more rapid improvement in barrier than solvent-dispersed physiologic lipids that
normalized lamellar body contents and lamellar bilayer architecture,\textsuperscript{20} and given the recent
report of CHILD syndrome skin lesions responding to topical simvastatin monotherapy in an
ointment base,\textsuperscript{33} monotherapy with topical statins for porokeratosis should be considered. In
addition, the kinetics of response to topical dual regimen versus monotherapy should be
studied.

In summary, topical cholesterol/lovastatin is an effective pathogenesis-directed treatment for
porokeratosis. While our experience treating a small series of patients supports the use of
topical cholesterol/lovastatin for porokeratosis treatment, larger randomized clinical trials will be
necessary to systematically evaluate the efficacy and safety of this therapy. \textit{In vitro} studies may
further increase our understanding of the importance of other end-product depletion in the
pathogenesis of this condition. In the interim, since cholesterol and lovastatin have a known
safety profile and are relatively inexpensive, the topical regimen provides a safe and effective
option of therapy in porokeratosis, including in cases with extensive skin involvement.


Abbreviations:

DSAP: disseminated superficial actinic porokeratosis
PPPD: porokeratosis palmaris et plantaris disseminate
LP: linear porokeratosis
SCC: squamous cell carcinoma
HMGCR: hydroxymethylglutaryl coenzyme A reductase
HMG-CoA: hydroxymethylglutaryl coenzyme A
FPP: farnesyl pyrophosphate
GGPP: geranylgeranyl pyrophosphate
WES: whole exome sequencing
gDNA: Genomic DNA
FFPE: formalin-fixed paraffin-embedded
HIDS: Hyper-IgD syndrome
Figure legends:

Figure 1: The Mevalonate pathway. The mevalonate pathway is an essential metabolic pathway that uses acetyl-CoA to produce sterols and isoprenoid metabolites that are essential for a broad range of metabolic processes. Genes previously found to be involved in porokeratosis are in bold. Asterisk marks genes in which mutations were found in the present study. Dashed arrows indicate multiple processes.

Figure 2: Disseminated superficial actinic porokeratosis. Clinical improvement of treated skin of FP100-1 with topical application of lovastatin/cholesterol. The patient shaved his arms prior to week 4 of therapy.

Figure 3: Porokeratosis palmaris et plantaris disseminata. Clinical improvement with topical application of cholesterol/lovastatin.

Figure 4: Linear porokeratosis. Clinical improvement with topical application of cholesterol/lovastatin.
Table 1: Clinical characteristics and genetic analysis of included patients.

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>Age</th>
<th>Age of porokeratosis onset</th>
<th>Porokeratosis subtype</th>
<th>History of skin cancer</th>
<th>Germline Mutation</th>
<th>Ref.</th>
<th>Non ref.</th>
<th>Somatic Mutation</th>
<th>Ref.</th>
<th>Non ref.</th>
<th># of reads in blood</th>
<th># of reads in affected tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>FP100-1</td>
<td>36</td>
<td>18 yo</td>
<td>DSAP</td>
<td>No</td>
<td>MVD c.70+5G&gt;A</td>
<td>8</td>
<td>10</td>
<td>44</td>
<td>42</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FP100-6</td>
<td>40</td>
<td>16 yo</td>
<td>PPPD</td>
<td>No</td>
<td>MVD c.70+5G&gt;A</td>
<td>18</td>
<td>12</td>
<td>30</td>
<td>31</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FP100-9</td>
<td>53</td>
<td>19 yo</td>
<td>PPPD</td>
<td>SCC</td>
<td>MVD c.70+5G&gt;A</td>
<td>16</td>
<td>15</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LP-1</td>
<td>20</td>
<td>Birth</td>
<td>LP</td>
<td>No</td>
<td>PMVK c.79G&gt;T, p.E27X</td>
<td>77</td>
<td>63</td>
<td>86</td>
<td>88</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LP-2</td>
<td>5</td>
<td>Birth</td>
<td>LP</td>
<td>No</td>
<td>PMVK c.329G&gt;A, p.R110Q</td>
<td>21</td>
<td>15</td>
<td>16</td>
<td>61</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*PMVK spans Chr1:154,897,208-154,909,484

Abbreviations: CN-LOH: copy-neutral loss of heterozygosity; DSAP: disseminated superficial actinic porokeratosis; CN-LOH= copy-neutral loss of heterozygosity; LP: linear porokeratosis; NA= not assessed; ND= not detected; PPPD: porokeratosis palmaris et plantaris disseminata; SCC: squamous cell carcinoma; ref: reference.