Petrolatum: Barrier repair and antimicrobial responses underlying this "inert" moisturizer

Tali Czarnowicki, MD,[a]* Dana Malajian, BA,[a,b]* Saakshi Khattri, MD,[a,c]* Joel Correa da Rosa, PhD,[a,d] Riana Dutt, ScB,[a,c] Robert Finney, MD,[e] Nikhil Dhingra, MD,[c] Peng Xiangyu, MSc,[a,c] Hui Xu, MSc,[a,c] Yeriel D. Estrada, BS,[c] Xiuzhong Zheng, MSc,[a] Patricia Gilleaudeau, NP,[a] Mary Sullivan-Whalen, NP,[a] Mayte Suaréz-Fariñas, PhD,[a,c,g,h,i] Avner Shemer, MD,[f] James G. Krueger, MD, PhD,[a] and Emma Guttman-Yassky, MD, PhD[a,c] *New York, NY, Philadelphia, Pa, and Tel Aviv, Israel*

Background: Petrolatum is a common moisturizer often used in the prevention of skin infections after ambulatory surgeries and as a maintenance therapy of atopic dermatitis (AD). However, the molecular responses induced by petrolatum in the skin have never been assessed.

Objective: We sought to define the cutaneous molecular and structural effects induced by petrolatum.

Methods: Thirty-six healthy subjects and 13 patients with moderate AD (mean SCORAD score, 39) were studied by using RT-PCR, gene arrays, immunohistochemistry, and immunofluorescence performed on control skin, petrolatum-occluded skin, and skin occluded with a Finn chamber only.

Results: Significant upregulations of antimicrobial peptides (S100A8/fold change [FCH], 13.04; S100A9/FCH, 11.28; CCL20/FCH, 8.36; PI3 [elafin]/FCH, 15.40; lipocalin 2/FCH, 6.94, human β-defensin 2 [DEFB4A]/FCH, 4.96; $P < .001$ for all) and innate immune genes (*IL6*, *IL8*, and *IL1B*; $P < .01$) were observed in petrolatum-occluded skin compared with expression in both control and occluded-only skin. Application of petrolatum also induced expression of key barrier differentiation markers (filaggrin and loricrin), increased stratum corneum thickness, and significantly reduced T-cell infiltrates in the setting of "normal-appearing" or nonlesional AD skin, which is known to harbor barrier and immune defects.

Conclusions: Petrolatum robustly modulates antimicrobials and epidermal differentiation barrier measures. These data shed light on the beneficial molecular responses of petrolatum in barrier-defective states, such as AD and postoperative wound care. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: *Petrolatum, moisturizer, occlusion, patch tests, antimicrobial peptides, innate immunity, atopic dermatitis, barrier, skin surgeries*

Petrolatum, available since 1872,[1] is a widely used moisturizer consisting mainly of long-chain aliphatic hydrocarbons.[2] It has been shown to decrease transepidermal water loss (TEWL) in healthy[3] and irritated[4,5] human skin. Although petrolatum is formally classified as an occlusive, it has also been described using the broader term "moisturizer," (a category that includes occlusives, as well as humectants and emollients), which reflects this therapeutic quality.[6]

Petrolatum has been proposed to protect against postambulatory surgical skin infections and is widely used after minor surgical procedures.[7] A large randomized trial of postoperative ambulatory surgery patients found petrolatum to be equivalent to bacitracin, a topical antibiotic commonly used in the prevention of infections.[8] Importantly, petrolatum rarely induces allergic contact dermatitis (ACD) reactions[9] and has never been reported to cause contact anaphylaxis, whereas bacitracin was shown to induce ACD in up to 13% of patients[10] and has caused contact anaphylaxis in several cases.[11-15] However, the molecular changes induced by petrolatum are unknown.

Lesional skin of patients with atopic dermatitis (AD) exhibits immune and barrier defects with increased penetration of small molecules.[16-19] This results in increased prevalence of ACD and microbial colonization/infection in the population with AD.[20,21] Despite similar bacterial colonization in patients with AD and those with psoriasis,[22] another common inflammatory skin disease, significantly higher rates of skin infection were observed only in patients with AD.[23] These differences were postulated to result in part from significantly lower antimicrobial peptide (AMP) responses in AD lesions compared with psoriatic lesions.[22,24] In adult and pediatric patients with AD, moisturizers have been shown to improve AD disease severity,[25] TEWL,[26] and

From [a]the Laboratory for Investigative Dermatology and [d]the Center for Clinical and Translational Science, The Rockefeller University, New York; [b]Columbia University College of Physicians and Surgeons, New York; the Departments of [c]Dermatology, [g]Population Health Science and Policy, and [h]Genetics and Genomics Science and [i]the Icahn Institute for Genomics and Multiscale Biology, Icahn School of Medicine at Mount Sinai, New York; [e]the Department of Dermatology, Jefferson Medical College, Philadelphia; and [f]the Department of Dermatology, Tel-Hashomer Hospital, Tel Aviv.

*These authors contributed equally to this work.

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Corresponding author: Emma Guttman-Yassky, MD, PhD, Department of Dermatology, Icahn School of Medicine at Mount Sinai Medical Center, 5 E 98th St, New York, NY 10029. E-mail: eguttman@rockefeller.edu.

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Abbreviations used
ACD: Allergic contact dermatitis
AD: Atopic dermatitis
AHR: Aryl hydrocarbon receptor
AMP: Antimicrobial peptide
DC: Dendritic cell
DEG: Differentially expressed gene
FCH: Fold change
FLG: Filaggrin
HBD2: Human β-defensin 2
H&E: Hematoxylin and eosin
IHC: Immunohistochemistry
LCN2: Lipocalin 2
LOR: Loricrin
PI3: Peptidase inhibitor 3/elafin
QC: Quality control
SC: Stratum corneum
TEWL: Transepidermal water loss

skin capacitance,[27] as well as to reduce rates of *Staphylococcus aureus* colonization.[28] Emollients have recently been shown to effectively prevent AD development in high-risk newborns.[29,30]

This study aims to uncover the molecular responses triggered by petrolatum application that can improve clinical and barrier measures, ultimately reducing infections in patients with barrier-disrupted conditions, such as AD, and after cutaneous surgeries. In 2 separate cohorts totaling 49 patients, we detected significant upregulation of key AMPs and innate immune genes in biopsy specimens from skin occluded with petrolatum compared with occlusion alone and control (or nonlesional AD) skin. Occlusion with petrolatum also resulted in altered epidermal structure and increased expression of terminal differentiation proteins, including filaggrin (FLG) and loricrin (LOR), which were particularly evident when petrolatum was applied to "normal-appearing" or nonlesional AD skin.

METHODS

Patients' characteristics and skin samples

This study included 2 cohorts under institutional review board–approved protocols. The first cohort included 29 patients (18 female/11 male patients; age, 19-62 years [median, 38 years]; 13 with AD/16 without AD; mean SCORAD score, 39). These patients had petrolatum ("White petrolatum"; Dynarex, Israel; NDC #67777-211-01) applied for 48 hours under occlusion with a Finn chamber, with biopsy specimens taken at 72 hours (24 hours after removal of petrolatum). A total of 2 biopsy specimens were obtained from this cohort: petrolatum-occluded and control (nonoccluded or nonlesional in patients with AD) skin. In patients with AD, all patches were applied on uninvolved skin; lesional/eczematous skin biopsies were not involved in this study. The petrolatum used in this study contained white petrolatum USP 100% wt/wt. White petrolatum often contains small amounts of antioxidants to prevent discoloration but does not contain antimicrobial preservatives.

To evaluate occlusion effects, as well as to determine the time point of maximal gene induction, we enrolled a second cohort including 20 healthy volunteers (8 female/12 male subjects; age, 18-80 years [median, 50.5 years]). These subjects had biopsy specimens taken from control skin, skin occluded with petrolatum under a Finn chamber, and skin undergoing occlusion alone (under a Finn chamber). For assessing the time point of maximal gene induction, the first 5 volunteers in the second cohort had 5 biopsies performed: control, occlusion-only and occlusion with petrolatum at 48 hours, and occlusion-only and occlusion with petrolatum at 72 hours skin. After analysis of preliminary kinetic data and to minimize the number of skin biopsies, the remaining 15 volunteers had only 3 biopsies: control skin, occlusion at 72 hours, and petrolatum at 72 hours (a flow chart of the 2 cohorts is shown in Fig 1).

Immunostaining and immunofluorescence

Immunohistochemistry (IHC) was performed on frozen tissue sections by using anti-human mAbs against S100A8/A9, lipocalin 2 (LCN2), CCL20, FLG, and LOR (see Table E1 in this article's Online Repository at www.jacionline.org). Immunofluorescence staining for neutral lipids with Nile Red was performed, as previously reported.[31,32] Epidermal thickness and positive cells per millimeter were quantified for IHC with ImageJ V1.42 software (National Institutes of Health, Bethesda, Md), and immunofluorescence was imaged with MetaView software (Visitron Systems, Puchheim, Germany).[33] For further details, see the Methods section in this article's Online Repository at www.jacionline.org.

Quantitative RT-PCR and gene arrays

RNA was extracted for RT-PCR and gene arrays with EZ-PCR Core Reagents (Life Technologies, Grand Island, NY), and custom primers were generated (see Table E2 in this article's Online Repository at www.jacionline.org for primers and probes).[16,24,34-38] Expression values were normalized to human acidic ribosomal protein *(hARP)*. Human HGU133Plus2.0 GeneChip probe arrays (Affymetrix, Santa Clara, Calif) were used for gene arrays.[16,24,34-38] Total RNA was extracted with the Qiagen miRNeasy Mini Kit (Qiagen, Valencia, Calif), and DNA was removed with the Qiagen RNAse-free DNAse Set. Total RNA (50 ng) was reverse transcribed and amplified with Ovation Whole Blood Solution from NuGen (San Carlos, Calif). The labeled target was fragmented and hybridized to probe arrays by using the Encore Biotin Module from NuGen. For further details, see the Methods section in this article's Online Repository.

Statistical analysis

The 2 previously described cohorts (cohort 1, 29 subjects; cohort 2, 20 subjects) were analyzed separately. Quality control (QC) of microarray gene expression was carried out with the standard QC metrics from Quality Control R package. Expression values from arrays and RT-PCR were modeled by using linear mixed-effect models from the *limma* and *nlme* packages in R, respectively. For microarray data, differentially expressed genes (DEGs) in petrolatum were identified by the significance of moderated *t* statistics after Benjamini-Hochberg adjustment for multiple hypotheses and with a fold change (FCH) of greater than 2. Analysis of RT-PCR gene expression consisted of fitting the mixed-effects model followed by pairwise comparisons among skin types: petrolatum-occluded, occluded-only, and normal skin (cohort 2). The significance of the differences was displayed in bar plots. The subgroup analysis for patients with and without AD (cohort 1) was carried out by including in the mixed-effects model skin type (petrolatum-occluded or normal skin), AD personal history (yes/no), and their interaction as fixed effects. A random intercept was adjusted for each subject. The same approach used for RT-PCR was applied to IHC counts (cohort 1) under the assumption of normality. Estimating contrasts by using restricted maximum likelihood assessed the significance of the differences between specified groups. Heat maps were built based on the McQuitty algorithm and Euclidean distances. More specific details can be found in the Methods section this article's Online Repository.

RESULTS

Our study included 2 cohorts. The first contained 29 subjects who had biopsy specimens taken after 72 hours from control skin (or nonlesional skin for those with AD) and skin occluded with petrolatum for 48 hours. The second cohort included 20 healthy volunteers with biopsy specimens taken