Degradation Mechanism Induced by Psoriasis in Human Fingernails: A Different Approach

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TO THE EDITOR

Nail psoriasis has a significantly negative impact on patient life quality (Dogra and Arora, 2014; Martínez-García et al., 2014).

This study is focused on understanding the degradation mechanism induced by psoriasis in human fingernails. The chemical structure, elemental composition, and surface morphology of healthy and psoriatic fingernails were compared using spectroscopic and imaging methods: attenuated total reflection-Fourier transform infrared spectroscopy, X-ray photoelectron spectroscopy, energy-dispersive X-ray spectroscopy, and scanning electron microscopy.

Fingernail clippings from 23 patients with psoriasis and 20 healthy donors were investigated (Supplementary Materials and Methods online) after obtaining written informed consent and approval by Ethics Committee of "Grigore T. Popa" University of Medicine and Pharmacy following Declaration of Helsinki protocols. Understanding the degradation mechanism can allow an early diagnosis, before high damage occurs. With noninvasive techniques, we identified differences in the molecular structure and elemental composition of keratin from psoriatic nails.

The molecular changes in the secondary structure of keratin were studied by analyzing the amide I region (1,700–1,600 cm^{-1}) in attenuated total reflection-Fourier transform infrared spectroscopy spectra; this region is sensitive to changes in secondary structures (Srisayam et al., 2014). After spectra deconvolution, the amide I regions of healthy and psoriatic nails (Figure 1a) were compared. The four components of the secondary structure were attributed to β-sheet, random coil, α-helix, and β-turns (Sakudo et al., 2009; Srisayam et al., 2014). Regardless of age or gender, a significant reduction of α-helix content coupled with an increase of β-sheet and random coil content was observed in psoriatic fingernails (Table 1). Nails are hard α-keratins, which abound in α-helix (Bragulla and Homberger, 2009), and a decrease in α-helix destabilizes the nail proteins (Sakudo et al., 2009). On the other hand, the β-sheet increase is due to keratin misfolding in amyloid-like fibrils—intermolecular β-sheets involved in protein aggregation (Balzani et al., 2012; Campioni et al., 2010). We can conclude that the damage produced by psoriasis in fingernails is associated with the α-β transition followed by a β-sheet-mediated protein aggregation. Moreover, the random coil increase is indicative of protein denaturation (Sakudo et al., 2009).

In addition, attenuated total reflection-Fourier transform infrared spectroscopy spectra showed absorption bands at 1,170 and 1,040 cm^{-1} (Figure 1b), which are specific to the S-O bonds of the cysteic acid unit. Increased band intensity in psoriatic fingernails indicates that certain changes also occur in the disulfide bridges (-S-S-) of cystine units. -S-S- bridges are degraded in psoriatic fingernails, first by reduction to thiol groups (−SH) and then by oxidation to sulfite groups ($-SO_3H$) (Supplementary Figure S1A-C online) (Oguri et al., 2012). This destabilizes the healthy nail matrix. Cysteic acid is normally present in healthy nails, as a part of sulfur amino acids (Ogura et al., 1962; Strzelczak et al., 2013), but a higher concentration of S-O bonds in psoriatic nails is indicative of nail matrix degradation (Figure 1c).

Energy-dispersive X-ray spectroscopy data (Supplementary Table S1 online) contain both elements with significant statistical differences ($P < 0.05$), such as oxygen (O), sulfur (S), and phosphorous (P), and elements without significant differences ($P > 0.05$), such as carbon (C) and nitrogen (N). The data confirm that the degradation process in psoriatic nails occurs by the conversion of disulfide bridges into sulfate groups, and/or into hydrogen sulfite and other volatile compounds (Supplementary Figure S1D online). This explains the decreased S content and the increased O content. The loss of S is sustained by the increased C/S ($P = 0.0318$) and N/S ($P = 0.0077$) ratios in psoriatic nails compared with healthy ones. Nail keratin also contains low concentrations of P (up to 0.18%), but an abnormally low content is more likely to be the result of a disease (Berndt and Kumar, 2009).

X-ray photoelectron spectroscopy results are consistent with energy-dispersive X-ray spectroscopy data, showing higher C and O contents and lower N and S contents in psoriatic nails (Figure 1d, Supplementary Table S2 online). X-ray photoelectron spectroscopy identifies the chemical structure by high-resolution spectra. The C1s high-resolution spectra, for both healthy and psoriatic fingernails (Figure 1e), were deconvoluted into five peaks, which can be seen in Supplementary Table S3 online. The concentrations of C-N and C-S bonds decrease in psoriatic nails, as the molecular structure of keratin is affected. As stated before, S is present because of the cystine, cysteine, and cysteic acid. A closer inspection of the S2p spectra (Figure 1f, Supplementary Table S3) confirms that the reduction of -S-S- bridges to −SH groups. Some of these are subsequently transformed into sulfite groups. The peaks for S=O and S-OH bonds, which indicate the presence of cysteic acid, were increased in psoriatic fingernails, whereas the S-S peak was decreased. In conclusion, the damages caused by psoriasis to the cystine disulfide bridges determine the appearance of a higher concentration of cysteic acid.

We also investigated healthy and psoriatic nails with scanning electron

Abbreviations: C, carbon; N, nitrogen; O, oxygen; P, phosphorous; S, sulfur

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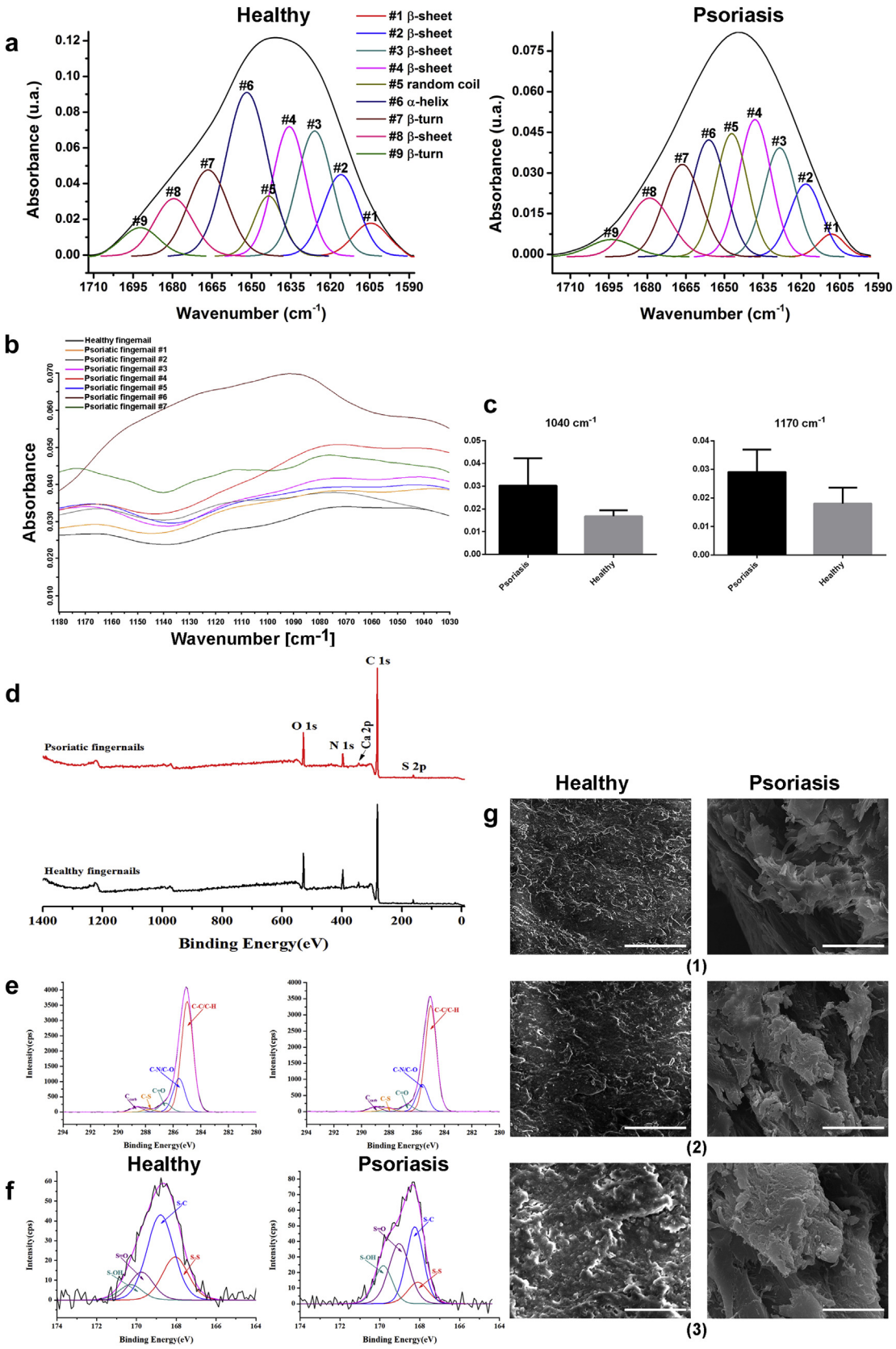

Figure 1. ATR-FTIR, XPS, and SEM results. **(a)** ATR-FTIR deconvolution of the amide I region in one healthy and one psoriatic fingernail. **(b)** ATR-FTIR regions of S-O bonds. **(c)** Statistical data resulted from the ATR-FTIR spectra of 1,040 and 1,170 cm^{-1} regions (represented as mean values). **(d)** XPS wide scans of one healthy and one psoriatic fingernail. **(e)** XPS high-resolution spectra of the C1s spectrum for one healthy and one psoriatic fingernail. **(f)** XPS high-resolution spectra of the S2p spectrum for one healthy and one psoriatic fingernail. **(g)** Selected SEM micrographs at different magnifications of the ventral layer of healthy and psoriatic fingernails (scale bar **(g.1)** = 100 μm, scale bar **(g.2)** = 50 μm, scale bar **(g.3)** = 20 μm). ATR-FTIR, attenuated total reflection-Fourier transform infrared spectroscopy; SEM, scanning electron microscopy; XPS, X-ray photoelectron spectroscopy.

Table 1. Average for secondary structural ratios of proteins in fingernails of healthy and psoriatic donors, of both males and females

	α-helix	β-sheet	β-turn	Random coil
Healthy nails (%)	29.72 ± 0.33	44.56 ± 0.31	16.75 ± 0.13	8.67 ± 0.15
Psoriatic nails (%)	15.25 ± 0.20	53.44 ± 0.44	16.37 ± 0.17	14.94 ± 0.24
Unpaired *t*-test (*P* value)	****(0.0001)	****(0.0001)	0.0972	****(0.0001)

The amide I region in attenuated total reflection-Fourier transform infrared spectroscopy spectra of male and female fingernails of both healthy donors and psoriatic patients was subjected to calculation for the secondary structural ratio (mean ± SEM), as described in Materials and Methods (see Supplementary Information online). The unpaired *t*-test was performed and a *P* value <0.05 was considered significant.

microscopy, which allows the correlation of different factors with the changes in surface morphology and can give unambiguous information about protein dynamics (Drummond and Allen, 2008). The selected images (Figure 1g) show that, compared with psoriatic fingernails, the ventral surface of healthy nails is smoother and more uniform with higher density, less roughness, and tight coherence, with only occasional separation of one or more corneocytes. This is a consequence of the higher content of disulfide bridges (Repka et al., 2002). In psoriatic fingernails, the ventral surface morphology changes quite drastically in uniformity and topography, with more irregular surface and higher roughness, possibly due to the broken disulfide bridges and keratin conformational changes.

All obtained results were complementary and consistently demonstrated that (i) psoriasis produces a significant decrease of α-helix, and an increase of β-sheet and random coil components; (ii) psoriasis causes the degradation of disulfide bridges of the cystine to thiol groups, some of which are subsequently transformed into sulfite groups or are completely degraded. These modifications destabilize the healthy nail matrix, which induces changes in surface morphology in terms of uniformity, density, and roughness. We can also conclude that the apparently beneficial effects of topical treatments last only for a short period of time because of the β-sheet-mediated protein aggregation in psoriatic nails. The aggregates are not broken down and the reconstruction of the initial keratin structure is not achieved.

The techniques used in our study could help in the development and optimization of noninvasive diagnostic methods and new treatments.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at http://dx.doi.org/10.1038/JID.2015.387.

REFERENCES

Balzani A, Pagnotta A, Montesi G, Gravante G, Nicoli F, Cervelli V. A case of psoriasis with secondary amyloidosis, associated symbrachydactyly of the hand and a transverse deficiency of the foot. Eur Rev Med Pharmacol Sci 2012;16:983–5.

Berndt T, Kumar R. Novel mechanisms in the regulation of phosphorus homeostasis. Physiology 2009;24:17–25.

Bragulla HH, Homberger DG. Structure and functions of keratin proteins in simple, stratified, keratinized and cornified epithelia. J Anat 2009;214:516–59.

Campioni S, Monsellier E, Chiti F. Why proteins misfold. In: Ramirez-Alvarado M, Kelly JW, Dobson CM, editors. Protein misfolding diseases: current and emerging principles and therapies. Hoboken, NJ: John Wiley & Sons; 2010. p. 3–20.

Dogra A, Arora AK. Nail psoriasis: the journey so far. Indian J Dermatol 2014;59:319–33.

Drummond SP, Allen TD. From live-cell imaging to scanning electron microscopy (SEM): the use of green fluorescent protein (GFP) as a common label. Methods Cell Biol 2008;88:97–108.

Martínez-García E, Arias-Santiago S, Valenzuela-Salas I, et al. Quality of life in persons living with psoriasis patients. J Am Acad Dermatol 2014;71:302–7.

Ogura R, Knox JM, Griffin AC, Kusuhara M. The concentration of sulfhydryl and disulfide in human epidermis, hair and nail. J Invest Dermatol 1962;38:69–75.

Oguri T, Schneider B, Reitzer L. Cysteine catabolism and cysteine desulfhydrase (CdsH/STM0458) in Salmonella enterica serovar typhimurium. J Bacteriol 2012;194:4366–76.

Repka MA, O'Haver J, See CH, Gutta K, Munjal M. Nail morphology studies as assessments for onychomycosis treatment modalities. Int J Pharm 2002;245:25–36.

Sakudo A, Kuratsune H, Kato YH, Ikuta K. Secondary structural changes of proteins in fingernails of chronic fatigue syndrome patients from Fourier-transform infrared spectra. Clin Chim Acta Int J Clin Chem 2009;402:75–8.

Srisayam M, Weerapreeyakul N, Barusrux S, Tanthanuch W, Thumanu K. Application of FTIR microspectroscopy for characterization of biomolecular changes in human melanoma cells treated by sesamol and kojic acid. J Dermatol Sci 2014;73:241–50.

Strzelczak G, Sterniczuk M, Sadło J, Kowalska M, Michalik J. EPR study of g-irradiated feather keratin and human fingernails concerning retrospective dose assessment. Nukleonika 2013;258:505–9.