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Topical Gentamicin for the Treatment of Genetic Skin Diseases

Anna M.G. Pasmooij[1]

Clinical application of topical gentamicin is a worthwhile option to investigate further for Nagashima-type palmoplantar keratosis and other genetic skin diseases caused by nonsense mutations. It is especially interesting to study gentamicin 1B because it may be more efficacious than other gentamicin components. Topical gentamicin has an acceptable safety profile, although prospective tracking of antibiotic resistance is warranted.

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Ohguchi et al. (2017) present interesting in vitro and in vivo findings regarding the use of topical gentamicin for the treatment of Nagashima-type palmoplantar keratosis (NPPK; OMIM. Johns Hopkins University, Baltimore, MD. MIM Number: 615598. http://www.ncbi.nlm.nih.gov/omim/). In this commentary, the article of Ohguchi et al. is highlighted, and gentamicin therapies are discussed in broader terms, for example, relating to the heterogeneity of gentamicin preparations and gentamicin's systemic toxicity. The possible utility of topical gentamicin for other genetic skin diseases is also discussed.

Disease characteristics of NPPK and current treatment options

Ohguchi et al. (2017) investigated gentamicin treatment in NPPK, an autosomal recessive disorder caused by mutations in *SERPINB7* (a member of the serine protease inhibitor superfamily). This palmoplantar keratoderma, first described by Nagashima in 1977, is characterized by well-demarcated erythematous hyperkeratosis extending onto the dorsal surfaces of the palms and feet and the Achilles tendon area (Figure 1). In some patients, a characteristic white spongy appearance has been observed after exposure of lesional skin to water. Mutations in *SERPINB7* are most frequent in Asians, with an estimated prevalence rate of NPPK of 1.2/10,000 in Japanese and 3.1/10,000 in Chinese Han individuals (Kubo et al., 2013). In the literature, eight distinct mutations (including nonsense, frame-shift, splice-site, and missense mutations) have been identified so far, with the nonsense c.796C>T (p.Arg266Ter) mutation in the last exon of *SERPINB7* being the most prevalent. To reduce hyperkeratosis, topical vitamin D3 and/or topical keratolytics, such as salicylic acid, urea, and adapalene, are administered. No curative therapy is available for NPPK, leading Ohguchi et al. (2017) to investigate gentamicin as a treatment option.

Gentamicin as treatment for NPPK

First, Ohguchi et al. (2017) transfected 293 cells with *SERPINB7* cDNA carrying the mutation and showed that gentamicin induced dose-dependent readthrough and expression of full-length SERPINB7 protein in these cells. Subsequently, gentamicin was tested in immortalized primary keratinocytes from a patient with NPPK who was homozygous for the c.796C>T mutation. The patient's cells produced full-length SERPINB7 protein after the addition of gentamicin to the medium. After obtaining these in vitro data, the investigators enrolled five patients with NPPK with c.796C>T mutations in an investigator-blinded, randomized, bilaterally controlled clinical study with 0.1% topical gentamicin ointment. A blinded investigator assessed hyperkeratosis, and scored it as improved in two out of five patients, whereas no differences in erythema were observed. This study provides encouraging data that justify further investigation of locally applied gentamicin for the treatment of NPPK due to the recurrent founder c.796C>T mutation.

Why did Ohguchi et al. investigate gentamicin for readthrough?

Gentamicin is a bactericidal antibiotic that possesses a broad antibacterial spectrum of action. Its activity includes Gram-positive bacteria such as *Staphylococcus* and Gram-negative microorganisms including *Escherichia coli*, *Klebsiella, Enterobacter*, *Pseudomonas*, and *Proteus*. This aminoglycoside is approved for the treatment of infections due to bacteria susceptibility to gentamicin, including bone infections, urinary tract infections, eye and ear infections, chest infections, bacteremia, septicemia, severe neonatal infections, and other systemic infections. Gentamicin binds to the prokaryotic 30S ribosomal subunit at the aminoacyl-tRNA acceptor site (A) on the 16S ribosomal RNA in the major groove at the site of the internal loop formed by the conserved residues A1408, A1492, and A1493. This induces a conformational change in the RNA by displacement of A1492 and A1493, thereby affecting protein synthesis by induction of codon misreading and inhibition of translocation (Wilson, 2014; Yoshizawa et al., 1998). Gentamicin also interacts with the small eukaryotic ribosomal subunit, but to a lesser extent than with the small prokaryotic ribosomal subunit.

The ability of gentamicin to interfere with mRNA proofreading has been exploited to treat genetic diseases that result from premature stop codons. Approximately 10% of genetic diseases are caused by nonsense mutations, in which a stop codon is introduced and

[1]*Department of Dermatology, Center of Expertise of Blistering Diseases, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands*

Correspondence: Anna M.G. Pasmooij, Department of Dermatology, Center of Expertise of Blistering Diseases, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9713GZ, Groningen, The Netherlands. E-mail: a.m.g.pasmooij@umcg.nl

Clinical Implications

- Topical gentamicin is a potentially useful treatment option to investigate for genodermatoses.
- Gentamicin, especially gentamicin B1, can induce readthrough of nonsense mutations.
- The systemic toxicity of gentamicin is anticipated to be low after topical administration.

the synthesis of the full-length protein is terminated prematurely. Gentamicin can cause cells to bypass stop codons, insert random amino acids, and express full-length proteins. Genetic skin diseases in which systemic gentamicin has been tested include cystic fibrosis and Duchenne muscular dystrophy (for review see Linde and Kerem, 2008). Topical gentamicin has also been administered previously to a patient with Hailey-Hailey disease, a rare inherited skin disorder that is characterized by blisters and erosions (Kellermayer et al., 2006). Kellermayer et al. (2006) found that topical gentamicin (0.1%) (1 mg/ml) was more effective in inducing remission in a patient with Hailey-Hailey disease carrying a premature stop mutation c.1402C>T in the *ATP2C1* gene than its comparator (5% boric acid and 2% salicylic acid).

Gentamicin: a mixture of different components

Gentamicin is a mixture of compounds that is a fermentation product of *Micromonospora purpurea*. It includes the major components gentamicins C1, C1a, C2, C2a, and a minor one C2b (Figure 2). Related substances, including sisomicin, garamine, gentamicin B, gentamicin B1, and 2-deoxystreptamine, are also formed in small amounts during fermentation (Stypulkowska et al., 2010). The US Pharmacopeial Convention specifies the composition of gentamicin as 25–50% gentamicin C1; 10–35% gentamicin C1a; and 25–55% gentamicin C2 + C2a, whereas the European Pharmacopoeia requires 20–40% gentamicin C1; 10–30% gentamicin C1a; and 40–60% gentamicin C2 + C2a + C2b. No specifications are provided for the related substances that are formed, and the amount of these related substances can thus vary between preparations. The major components of gentamicin differ with regard to the degree of methylation in the 2-amino-hexose (purpurosamine) ring, and antimicrobial potencies as well as toxicities are different (see the paragraph below). A recent publication by Baradaran-Heravi et al. (2017) showed that the components in gentamicin also have varying nonsense mutation suppression activities. Gentamicin B1 has major nonsense mutation suppression activity, whereas the very close structural analog gentamicin B lacks readthrough activity. The previously observed variation in effects of gentamicin in the treatment of genetic diseases carrying premature termination codons (Linde and Kerem, 2008) could possibly be due to varying contributions of the gentamicin B1 compound.

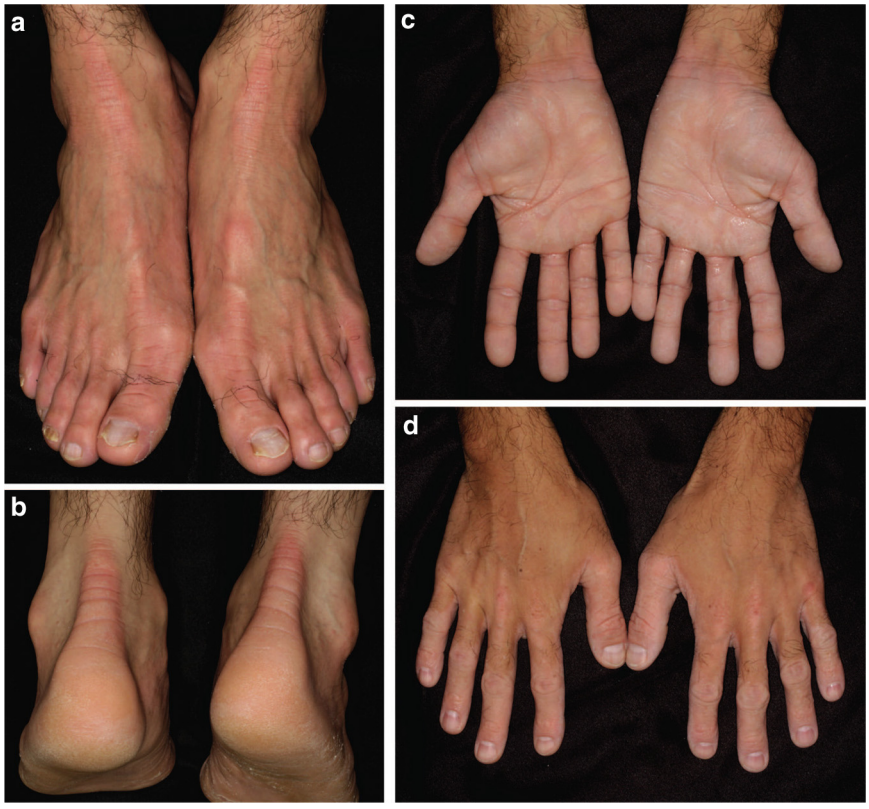

Figure 1. Clinical appearance of Nagashima-type palmoplantar keratosis. (**a**, **b**) "Transgrediens" of the reddish mild hyperkeratosis on the dorsal surfaces of the feet and Achilles tendon area. (**c**, **d**) Whitish spongy changes on the hands after 10 minutes of water exposure. (**d**) "Transgrediens" on the dorsal surfaces of the hands. Republished with permission from Kubo (2014).

Adverse events associated with systemic administration: ototoxicity and nephrotoxicity

The most frequently reported adverse events associated with aminoglycosides after systemic administration are toxicity to the ear (ototoxicity), with 11% of people who receive aminoglycosides experiencing damage to the inner ear, and kidney damage (nephrotoxicity) in 10–25% of recipients. These toxicities occur more frequently in patients who experience prolonged exposure to serum gentamicin trough concentrations of greater than 2 μg/ml. In addition, genetic analysis of individuals who are hypersensitive to deafness due to aminoglycosides has led to the identification of mutations in the mitochondrial small ribosomal RNA gene 1555A>G