Topical Gentamicin for the Treatment of Genetic Skin Diseases

Anna M.G. Pasmooij

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 in NPPK 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.

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 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 aminocyl-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...
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.

**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.
and 1494C>T that increase drug binding and render the ribosomal decoding site hypersusceptible to aminoglycoside-induced mistranslation and inhibition of protein synthesis (Hobbie et al., 2008). The toxicities of different gentamicin components vary, as was shown by Kobayashi et al. (2003) in a study in Hartley guinea pigs in which daily subcutaneous injections of the major components of gentamicin were administered. The results showed that the C2 compound produced the strongest toxic effects to the auditory portion of the inner ear (cochleotoxicity) and central part of the bony labyrinth in the inner ear (vestibulotoxicity), whereas C1a produced the weakest cochleotoxic effect, but considerable vestibulotoxic effects. Concerns regarding ototoxicity and nephrotoxicity are not likely after topical administration to the skin because systemic exposure would be relatively low, consistent with the lack of clinical signs of systemic toxicity in the study of Ohguchi et al. (2017). Although the levels of gentamicin in blood were not measured, the authors argued that the daily dose of 0.1% topical gentamicin ointment was much lower than in previously reported clinical studies. In future clinical trials for NPPK and other genetic skin diseases, it would be advisable to measure serum gentamicin concentrations to determine the level of systemic exposure after topical application.

Adverse events associated with topical administration: erythema and pruritus
In the study of Ohguchi et al. (2017), one of the five patients developed pruritic erythema and vesicles after 2 weeks of gentamicin treatment. This possibility of irritation (erythema and pruritus) is mentioned in the FDA labeling of 0.1% gentamicin sulfate cream. The label indicates that irritation usually does not require discontinuation of treatment, and that it occurs in a small percentage of cases. No other adverse events are noted in the FDA labeling, except for possible photosensitivity in several patients (although a response could not be elicited in these patients by reapplication of gentamicin sulfate after exposure to UVR). Adverse events after topical administration of gentamicin can therefore be considered to be relatively mild.

Another aspect to consider is the potential risk of antibiotic resistance after chronic gentamicin exposure, although the risk after topical administration is largely unknown. However, studies in which gentamicin was provided prophytactically in catheter irrigation solutions have reported an increased gentamicin resistance. Although the method of administration was different in these studies, these findings suggest that chronic exposure to topical gentamicin could enhance bacterial resistance (Nessim and Jassal, 2012). Moreover, the guideline for the treatment of acne of the European Dermatology Forum states that because of the serious concerns regarding the risk of developing antibiotic resistance, topical monotherapy with antibiotics is generally not recommended (Nast et al., 2016). Thus, prospective tracking of the potential development of antibiotic resistance is warranted in clinical trials studying topical gentamicin treatment.

Other studies with gentamicin in genetic skin diseases
Besides the work of Kellermayer et al. (2006) for Hailey-Hailey disease, the possible use of gentamicin has been investigated in other genetic diseases that involve the skin, including dystrophic epidermolysis bullosa, a severe blistering disorder of the skin and mucosa caused by mutations in the type VII collagen gene COL7A1 (Baradaran-Heravi et al., 2017; Woodley et al., 2017), and xeroderma pigmentosum group C (Kuschal et al., 2015), an autosomal recessive disorder in which the DNA repair is affected, thereby leading to a high occurrence of marked photosensitivity and increased occurrence of skin cancers. Woodley et al. (2017) performed a double-blind, placebo-controlled pilot trial with topical and intradermal gentamicin in five patients with recessive dystrophic epidermolysis bullosa with nonsense mutations. The topical arm tested 0.1% gentamicin ointment or placebo application three times daily at two open erosion sites for 2 weeks. The intradermal arm tested daily intradermal injection of gentamicin solution (8 mg) or placebo into two intact skin sites for 2 days in four of five patients. Topical gentamicin corrected dermal-epidermal separation, improved wound closure, and reduced blister formation. The authors concluded that gentamicin therapy may provide a readily available treatment for patients with recessive dystrophic epidermolysis bullosa with nonsense mutations. A follow-up trial is currently ongoing in which intravenous injection is investigated with the aim to treat all patients’ skin wounds simultaneously (ClinicalTrials.gov Identifier: NCT03012191).

Conclusions
Clinical application of topical gentamicin is a worthwhile option to investigate further for NPPK and for other genetic skin diseases caused by nonsense mutations. It may be especially interesting to study gentamicin B1 because gentamicin B1 may be more efficacious than other gentamicin components. Topical gentamicin has an acceptable safety profile. However, measuring systemic levels of gentamicin and prospective tracking of the potential
development of antibiotic resistance is advised for future clinical trials.

CONFLICT OF INTEREST
The author states no conflict of interest.

REFERENCES


See related article on pg 864

Targeting the Plasticity of Psoriasis

Jack L. Arbiser1,2 and Justin Elsey1

Psoriasis is a common inflammatory condition found in 1–2% of the population. The greatest advances in psoriasis treatment have occurred in patients with severe psoriasis, moving from systemic small molecules including methotrexate, cyclosporine, and retinoids to targeted agents against psoriasis-associated cytokines, such as TNF-α, IL-12, IL-23, and IL-17. Although the new biologics do not have the same adverse effects as the systemic drugs, they do predispose to systemic infections (and perhaps cancer), and they are extremely expensive. The focus on biologic therapies has been accompanied by a relative neglect of small molecules, which can be used either topically or systemically. No small molecule has had the same adverse effects as the systemic drugs, they do predispose to infections in peritoneal dialysis patients using topical gentamicin exit-site prophylaxis: a report of two cases. Perit Dial Int 2012;32:339–41.


Genetic, epidemiologic, and clinical studies have provided a vast amount of new knowledge about psoriasis in the 21st century. We now know that psoriasis is not one disease, but many that share common phenotypes (Liang et al., 2017). We also know the major cytokine drivers of psoriasis, including TNF-α, IL-17, and IL-23 (Guttman-Yassky et al., 2008; Nakajima et al., 2011; Xing et al., 2016). We know that both keratinocyte and lymphocyte intrinsic factors can mediate psoriasis (Wrone-Smith and Nickoloff, 1996), and that the effects of this interaction are not limited to the skin but are associated with generalized vascular inflammation, leading to an increased incidence of cardiovascular disease (Langan et al., 2012). We know that transcriptional factors in both lymphocytes and keratinocytes mediate psoriasis (loss of AP-1, Psors4) (Park et al., 2009; Zenz et al., 2005), gain of function of NF-κB (lymphocytes and keratinocytes) (Stuart et al., 2015), gain of function of STAT3 and STAT5 (keratinocytes, lymphocytes) (Sano et al., 2005), RORγT (lymphocytes) (Massot et al., 2014) (Figure 1). Finally, we also know that clinical and histologic phenotypes of psoriasis are mediated by specific transcription factors and cytokines (hyperkeratosis, loss of specific AP-1 subunits, NF-κB activation), redness (nicotinamide adenine dinucleotide phosphate-oxidase driving angiopoietin-2) (Hara-Chikuma et al., 2015; Perry et al., 2006), and loss of barrier function due to impaired ceramide generation and metabolism to sphingosine 1-phosphate (Arbiser et al., 2017).

Psoriasis is a moving target. Patients may have initial good responses and then have flares. Loss of response can be due to tachyphylaxis (topical steroids), poor delivery (all topical agents), antibodies against biologics, and up-regulation of compensatory signaling pathways as a response to therapy. Thus, novel treatments for psoriasis are needed.

Fuhrman et al. (2018) tested the efficacy of systemic PRN694, a potent inhibitor of ITK and RLK tyrosine kinases, in two murine models of psoriasis, the CBA/Caj K14 RAC1 and the topical imiquimod model. They found this small molecule to have preferential...