Aminoglycosides as Potential Pharmacogenetic Agents in the Treatment of Hailey–Hailey Disease

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TO THE EDITOR

The clinical use of aminoglycoside antibiotics lies in their antimicrobial activity due to their ability to inhibit bacterial translation. However, recent studies have also shown that aminoglycosides have the innate potential to induce readthrough of nonsense mutations in human cells. Consequently, this group of antibiotics has been experimentally utilized as potential pharmacogenetic agents to reverse the effects of pathogenic nonsense mutations in various human genetic disorders. Several *in vitro* and clinical studies gave promising results (Clancy *et al.*, 2001; Sleat *et al.*, 2001; Helip-Wooley *et al.*, 2002; Keeling and Bedwell, 2002; Wilschanski *et al.*, 2003; Aguiari *et al.*, 2004; Howard *et al.*, 2004). Nevertheless, the ability of aminoglycosides to induce readthrough of stop mutations has not yet been demonstrated for any of the genodermatoses. This oversight has occurred in spite of the fact that the therapeutic concentration of topical gentamicin, for instance, is 100-fold higher than that of the recommended serum concentrations. Additionally, the usual concern for aminoglycoside side effects (such as hearing loss and renal insufficiency) is generally not an issue during their topical application as long as the epidermal basal membrane is intact.

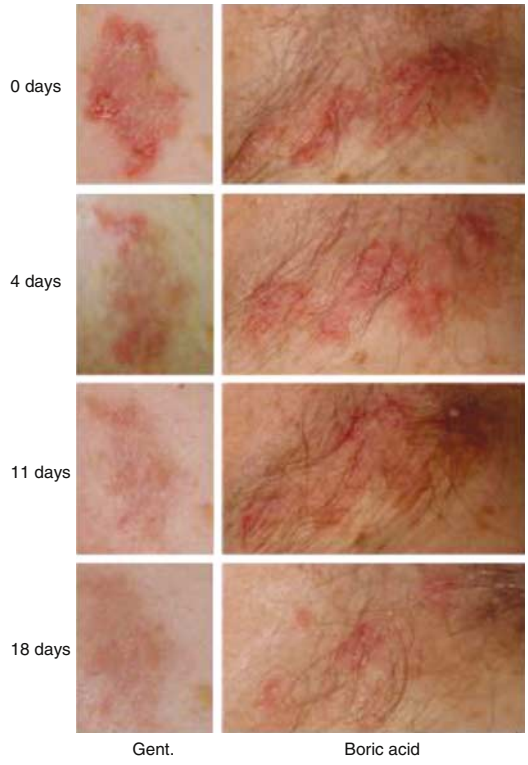

Figure 1. Topical gentamicin induces early remission in HHD. The study was conducted according to the Declaration of Helsinki principles and the medical ethical committee of the University of Pécs approved all described studies. Gentamicin (0.1%) (1 mg/ml) was applied twice topically to a submammary skin eruption (left column). The effect of this treatment was compared to a topical preparation containing 5% boric acid and 2% salicylic acid, which has been previously used successfully in the volunteer patient (right column). The patient carries the already reported 1402C>T mutation of *ATP2C1*, leading to a UGA premature stop mutation (R468X) (Hu *et al.*, 2000) (case report under preparation). The proband reported resolution of constitutional symptoms by day 2 and reached complete healing of the acute eruption between days 7 and 10 of gentamicin treatment. On the contrary, the area treated with the boric acid/salicylic acid preparation healed significantly slower with constitutional symptoms present until days 10–12 of treatment, and possessed small patches of erythema even by day 18 of therapy.

Hailey–Hailey disease (HHD, MIM# 169600) or chronic benign familial pemphigus is a blistering skin disorder that has been linked to mutations in the *ATP2C1* gene encoding the human secretory pathway Ca^{2+}/Mn^{2+} ATPase (hSPCA1) (Hu *et al.*, 2000; Sudbrak *et al.*, 2000). More than 80 pathogenic *ATP2C1* mutations have been reported in HHD patients, among which ~20% is a base substitution that causes a premature stop mutation that results in the synthesis of a truncated form of hSPCA1 (Foggia and Hovnanian, 2004). In this study, we addressed whether topical aminoglycosides may be beneficial for the treatment of HHD patients carrying nonsense mutations. Antibiotics in general have been among the treatment repertoire of HHD due to the observation that dermal infections can exacerbate the associated rash (Burge,

Abbreviations: HHD, Hailey–Hailey disease; hSPCA1, human secretory pathway Ca^{2+}/Mn^{2+} ATPase

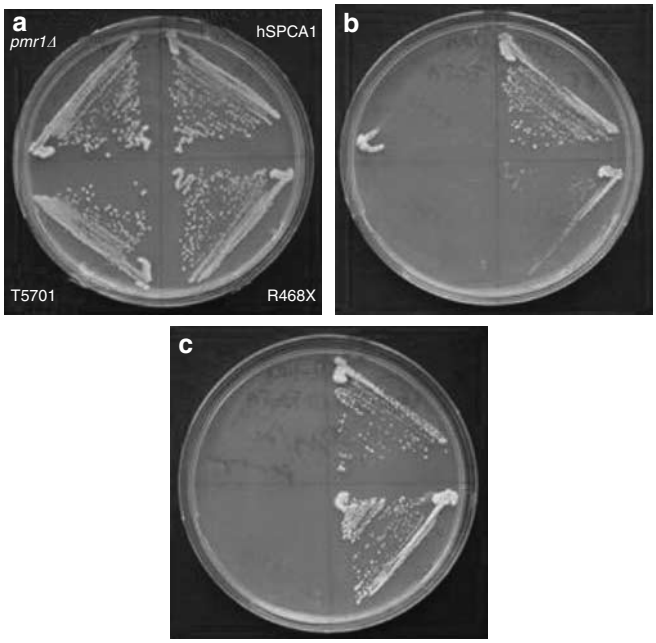

Figure 2. Paromomycin promotes the growth of a PMR1-defective yeast strain expressing hSPCA1-R468X. The *pmr1*Δ yeast strain YDB279 (Miseta *et al.*, 1999) is unable to grow in calcium-depleted growth media (such as media containing the chelating agents EGTA or BAPTA). This phenotype is complemented by the expression of hSPCA1 and this yeast model system has proved to be useful for functional testing of *ATP2C1* mutations (Ton and Rao, 2004). We introduced the 1402C>T mutation into a yeast expression plasmid harboring *ATP2C1* (a generous gift from Dr Rajini Rao) using the QuickChange site-directed mutagenesis kit (Stratagene) using primers DB2561 (5′-GCT GTT AAG TGT GTA CAC TGA ACA CAG CAG GAC-3′) and DB2562 (5′-GTC CTG CTG TGT TCA GTG TAC ACA CTT AAC AGC-3′). The *pmr1*Δ yeast strain YDB0279 was transformed with the plasmids expressing wild-type hSPCA1, hSPCA1-R468X, or hSPCA1-T570I. Growth of the strains was evaluated on yeast minimal media (SM-URA, 2% dextrose, 40 mM Mes-Tris, pH 6.5) (**a**) and the same media supplemented with 2 mM EGTA (**b**) or 2 mM EGTA + 50 μg/ml paromomycin (**c**). While only the strain expressing wild-type hSPCA1 could grow well on 2 mM EGTA, paromomycin stimulated the growth of the *pmr1*Δ strain expressing the hSPCA1-R468X mutant, but not the strain expressing the hSPCA1-T570I mutant. These results suggest that paromomycin can functionally reverse the effects of the R468X mutation by inducing readthrough of the UGA nonsense mutation.

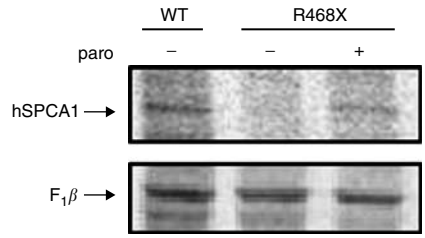

Figure 3. An increase in full-length hSPCA1 protein can be detected in yeast cells grown in the presence of aminoglycosides. A *pmr1*Δ yeast strain that expressed hSPCA1-R468X was grown in the presence (+) or absence (−) of paromomycin for 18 h. Cultures were then metabolically labeled with Tran ^{35}S-label (ICN Pharmaceuticals) for 40 minutes and cell extracts were immunoprecipitated using a rabbit polyclonal antibody to the carboxyl terminus (amino acids 720–919) of hSPCA1 (Santa Cruz Biotechnology, Inc.) and subjected to SDS-PAGE. Immunoprecipitated proteins were visualized by PhosphorImager analysis (GE Healthcare). While the truncated protein cannot be detected with this antibody, an increase in full-length hSPCA1 protein (115 kDa) was detected in yeast cells harboring the hSPCA1-R468X plasmid treated with 100 μg/ml paromomycin (+) compared to an untreated control strain (−). Besides hSPCA1, we also immunoprecipitated the beta subunit of the mitochondrial F1-ATPase beta subunit (indicated as F1β) from lysates of each strain. This control shows that total protein synthesis is not significantly increased in the presence of paromomycin.

1992). Indeed, staphylococci and other microbes may induce IL-6 expression in keratinocytes and consequently decrease hSPCA1 expression in an autocrine fashion; this process has been implicated in the exacerbation of symptoms in HHD (Sasaki *et al.*, 2003; Mayuzumi *et al.*, 2005). However, prior to recognizing the genetic background of HHD, gentamicin has specifically been found to be part of the optimal treatment regimen for some patients (Galimberti *et al.*, 1988). Indeed, we found that topical gentamicin caused remission in a volunteer HHD patient carrying an already reported UGA nonsense mutation (R468X) (Hu *et al.*, 2000) more than 10 days earlier than topical boric acid/salicylic acid therapy (Figure 1). Boric acid at the administered concentration (5%) is bactericidal against staphylococci and is comparable to gentamicin in its efficacy for the treatment of chronic otitis media (Benson, 1998; Moshi *et al.*, 2000). However, to our knowledge, boric acid does not affect readthrough.

As our elderly patient did not consent to a repeat skin biopsy (a diagnostic one has been performed for her several years before), we decided to test our new therapeutic approach at the molecular level in the eukaryotic *Saccharomyces cerevisiae* model of HHD. *ATP2C1* is an ortholog of the yeast *PMR1* gene (Kellermayer, 2005). hSPCA1 fully complements the phenotypes of PMR1-defective (*pmr1*Δ) yeast (Ton *et al.*, 2002) and heterologous expression of mutant *ATP2C1* in *pmr1*Δ *S. cerevisiae* cells has proved to be a useful screening method to address consequent functional disturbances of hSPCA1 (Ton and Rao, 2004). Additionally, the *pmr1*Δ yeast model system was shown to be valuable in understanding the potential pharmacomechanisms of therapeutic agents, such as FK506, for HHD (Szigeti and Kellermayer, 2004). Consequently, PMR1-deficient *S. cerevisiae* has proved to be a valuable model organism for HHD.

We introduced the 1402C>T mutation into *ATP2C1* in a yeast expression plasmid and transformed a *pmr1*Δ yeast strain with the construct. We found that paromomycin, an aminoglycoside capable of inducing efficient readthrough in yeast, stimulated growth of the *pmr1*Δ yeast strain expressing hSPCA1-R468X when compared to the same yeast strain expressing hSPCA1 with a pathogenic missense mutation (Figure 2). Furthermore, an increase in full-length hSPCA1 protein could be detected by immunoprecipitation of radiolabelled cell extracts when yeast

cells carrying a plasmid expressing hSPCA1-R468X were grown in the presence of paromomycin (Figure 3).

In conclusion, this study addresses topical aminoglycoside therapy in a genodermatosis with the objective of inducing readthrough of a pathogenic nonsense mutation. Topical gentamicin was found to be far more effective in inducing remission in a HHD patient carrying a premature stop mutation than an accepted topical disinfectant. Observations in a yeast model system of HHD supported the clinical findings that topical aminoglycosides may be beneficial therapeutic agents for patients harboring *ATP2C1* premature stop mutations. These findings will have to be addressed in the skin or keratinocytes of HHD patients harboring *ATP2C1* nonsense mutations to conclusively show that topical aminoglycosides can induce readthrough of premature stop mutations in the epidermis. However, our initial clinical and molecular observations highlight the potentially great value of topical aminoglycosides in the treatment of genodermatoses.

CONFLICT OF INTEREST

The author states no conflict of interest.

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