Article is available online at http://www.webio.hu/por/2006/12/4/0234

ARTICLE

Lithium Suppresses Epidermal SERCA2 and PMR1 Levels in the Rat

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Autosomal dominant mutations in the genes encoding the calcium ATPases SERCA2 and PMRI/SPCA1 cause the genodermatoses Darier disease (DD) and Hailey-Hailey disease (HHD), respectively. Recent observations indicated that the level of the pathogenic proteins greatly decreases in the affected areas of the epidermis in these disorders. Here we addressed how lithium, a recognized exacerbating factor in Darier disease, affects the epidermal expression of SERCA2 and PMR1/SPCA1 in the rat as a model. Standard histologic and immunohistochemical methods were utilized in 3 lithium-treated and 3 control animals. A significant suppression of epidermal SERCA2 and PMR1 levels were observed as a result of lithium therapy in addition to marked qualitative and quantitative changes in the stratum corneum and the granular layer of the epidermis in the treated animals. Our findings suggest that exacerbating factors in calcium ATPase disorders of the skin suppress epidermal SERCA2 and PMR1 levels, further decreasing the already haploinsufficient protein expression to a potentially critical level in Darier disease and Hailey-Hailey disease, respectively. Lithium therapy should specifically be avoided not only in Darier disease, but Hailey-Hailey disease as well. (Pathology Oncology Research Vol 12, No 4, 234–236)

Key words: Hailey-Hailey disease, Darier disease, lithium, rat, SERCA2, PMR1

Introduction

Darier disease (DD, keratosis follicularis, MIM#124200) and Hailey-Hailey disease (HHD, benign familial pemphigus, MIM#169600) are the only recognized human autosomal dominant Ca^{2+} ATPase disorders. Pathogenic mutations in the *ATP2A2* gene encoding SERCA2 (sarco/endoplasmatic-reticulum Ca^{2+}-transport ATPase isoform 2) have been revealed in DD, while mutations in the *ATP2C1* gene encoding the human secretory pathway Ca^{2+}/Mn^{2+} ATPase (hSPCA1) have been shown to occur in HHD. Only the epidermis is affected in these genodermatoses, where haploinsufficiency appears to be the primary mechanism of the dominant inheritance. Yet, none or only a slight reduction of SERCA2 or SPCA1 levels can be observed in the non-affected epidermis of DD or HHD patients compared to the skin of healthy individuals. On the contrary, the expression of both proteins significantly decreases in the epidermal areas where the rash occurs, usually as a consequence of exacerbating factors such as UVB, heat, friction and infection. It has been shown in cultured keratinocytes that *ATP2A2* and *ATP2C1* expression is suppressed following UVB stimulation, a recognized exacerbating factor in both DD and HHD. These results indicate that aggravating factors in the autosomal dominant Ca^{2+} ATPase disorders induce suppression of the already haploinsufficient *ATP2A2* and *ATP2C1* transcription. Consequently, the amount of SERCA2 and SPCA1 may decline to a critical point in the already haploinsufficient epidermis of DD and HHD patients, inducing the pathogenesis of the acantholytic changes characteristic for these disorders.

In this study we addressed the effects of oral lithium exposure, an exacerbating factor in DD, on epidermal SERCA2 and PMR1 (plasma membrane-related ATPase 1) levels in the rat. PMR1 is the ortholog of human SPCA1 and the former designation of the protein is used commonly in model organisms.

Received: June 19, 2006; *accepted:* Oct 21, 2006

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Materials and Methods

Male Wistar rats of 100-130 g received either a regular diet and tap water (control group, 3 animals), or regular diet and tap water containing 15 mmol/l LiCl (lithium-exposure group, 3 animals) for 12 days. The animals were sacrificed through prolonged ether narcosis. A 1x1 cm skin sample was cut from their back and placed into 4% formaldehyde for fixation.

Histological evaluation. 3- to 4-μm thick sections of skin from the lithium-exposed and control animals were stained with hematoxylin-eosin (H&E) according to standard methods and evaluated for histological features such as general architecture, thickness of the different epidermal layers, pattern and intensity of staining. The thickness of the epidermal layers was measured with an ocular scale at 400x magnification and then multiplied by the appropriate conversion factor. Ten separate reference points were used in every 200 micrometers along the skin surface of each animal involved in the study. The mean values were calculated from the collected data.

Immunohistochemical staining. Immunohistochemical staining was performed with a DAKO autostainer (DAKO, Glostrup, Denmark) using a standard indirect avidin-biotin peroxidase detection method. Sections were incubated at 56°C overnight on silanized slides, de-waxed and hydrated through diluted ethanol solutions to distilled water. Using a pressure cooker for 15 min all of the sections underwent antigen retrieval in 10 mM citrate buffer (pH=6.0). After cooling for 20 minutes at room temperature sections were stained with antibodies against PMR1 (H-200, Cat#sc-5548) and SERCA2 (C-20, Cat#sc-8094) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA; 1:50 dilution). The evaluation of routine H&E sections, the immunohistochemical results and measurements of the granular layer were independently performed and reviewed by two of the authors (NS and EK).

Results

Histological examination of the skin excised from the lithium-exposed animals revealed marked qualitative and quantitative changes in the stratum corneum and the granular layer of the epidermis compared to the control specimens. A significantly thicker horny layer (thickness ranging from 32 to 64 μm, mean 45.2 μm) could be detected in the exposed animals than in the control group (thickness ranging from 7 to 48 μm, mean 22.1 μm). Similar, but less pronounced changes occurred in the living cellular layers of the epidermis (Li^+ exposure: thickness ranging from 16 to 42 μm, mean 28 μm vs. control: thickness ranging from 14 to 48 μm, mean 22.4 μm). However, the stratum granulosum was not only thicker (ranging from 5 to 15 μm, mean 11.4 μm, vs. 0-7 μm, mean 3.18 μm), but also involved more cell layers in the Li^+-exposed animals. The keratohyaline granules seemed coarser and demonstrated less crisp staining than the granules present in the epithelial cells of the untreated animal specimens (*Figures 1a,d*). Differences in the epidermis between the two groups could also be demonstrated by altered immunohistochemical expression of the two Ca^{2+} ATPases studied. PMR1 and SERCA2 expression was clearly present in approximately 40% of the squamous epithelial cells of control animals (*Figures 1b,c*). The positive staining of the skin was mainly in the basal layer and the stratum spinosum, but scattered expression could also be observed in the granular layer. On the contrary, PMR1 and SERCA2 staining completely disappeared in the lithium-exposed animals (*Figures 1e,f*).

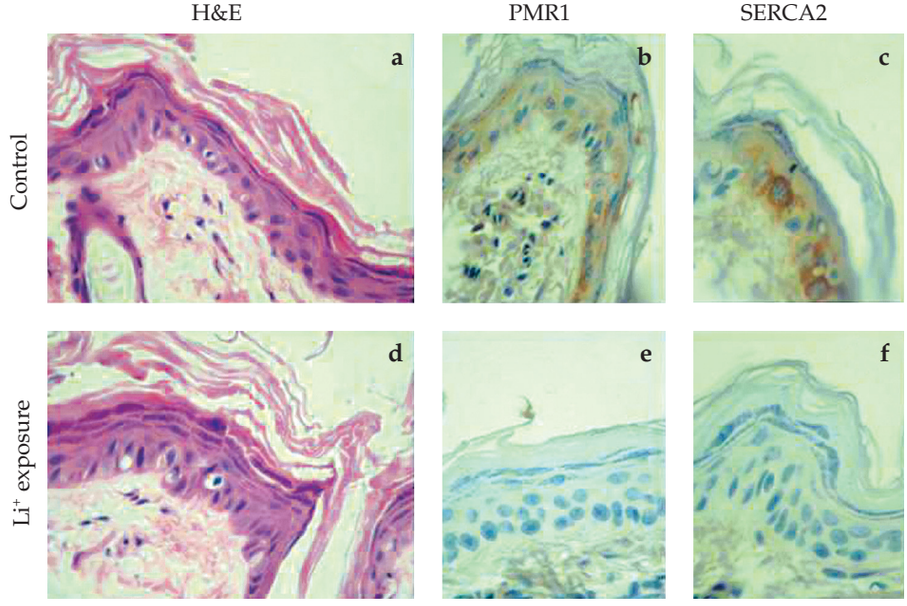

Figure 1. *Comparison of control* ***(a)*** *and lithium-exposed* ***(d)*** *skin reveals prominent changes in the horny and granular layers (H&E). As a result of lithium treatment, diminished PMR1 (****b*** *and* ***e****) and SERCA2 (****c*** *and* ***f****) expression of the epithelial cells could be observed. x400*

Discussion

Overexpression of keratins associated with keratinocyte hyperactivation has been observed in Atp2a2+/- mice, and dyskeratosis and hyperkeratosis are histological features of DD. However, *ATP2A2* haploinsufficiency does not result in similar dermal symptoms in mice as in DD, but leads to squamous cell tumors. Nevertheless, murine epidermal Ca^{2+} homeostasis is similar to that of humans. Consequently, observations in rodent skin regarding SERCA2 and PMR1 may be useful for understanding DD and HHD pathophysiology. In this study we observed that lithium exposure in rats induced a reduction in keratinocyte SERCA2 expression and a granular layer expansion with coarser and larger keratohyaline granules. This result is in alignment with the observations in Atp2a2+/- mice where hyperkeratosis occurred as a result of haploinsufficient SERCA2 levels. However, it should be noted that it cannot be discerned from our observations weather the morphological changes in the epidermis of the Li^+-treated animals are a direct consequence of SERCA2 and/or PMR1 suppression, or are due to other mechanisms initiated by Li^+. Nevertheless, the finding that lithium induces a reduction in the epidermal levels of SERCA2 and PMR1 is consistent with earlier observations revealing that exacerbating factors in human keratinocytes and epidermis cause an inhibition of *ATP2A2* and *ATP2C1* transcription. Our results suggest that lithium exacerbates the symptoms of DD through reducing the epidermal levels of SERCA2 to a critical point, promoting the acantholytic and other histological disturbances of the disorder. Such an effect of lithium has not been recorded yet in HHD despite of the fact that lithium suppressed PMR1 levels in our animal model. The reason for this may be that psychiatric symptoms are not associated with HHD in contrast to DD. Nevertheless, our observations indicate that lithium therapy should be avoided not only in DD, but HHD as well in case incidental psychiatric symptoms may occur. The conclusion of this report can provide an explanation for the effectiveness of translational read-through induction in HHD patients harboring nonsense *ATP2C1* mutations too. Indeed, if the expression of SPCA1 critically decreases in the affected areas of HHD skin, a small induction of *ATP2C1* nonsense mutation read-through during translation may promote earlier recovery by elevating the levels of SPCA1 in the areas of the rash.

Li^+ has been observed to exacerbate several dermal disorders. Putative, diverse pathogenic mechanisms have been implicated (inhibition of cAMP and inositol accumulation, inhibition of prostaglandin synthesis, increasing the number of circulating neutrophils and inducing lysosomal leukocyte release). Additionally, Li^+ has been shown to alter cellular Ca^{2+} homeostasis in yeast through inhibiting phosphoglucomutase activity, but similar effects could not yet be confirmed in mammals. Consequently, while Li^+ may influence a wide variety of cellular mechanisms, it is hard to perceive which could be the key to its effects on epidermal Ca^{2+} ATPase expression. Further molecular studies will be required in this respect.

Acknowledgements

The authors would like to thank Mária Kneif for her outstanding assistance. A.M. was supported by OTKA T-038144.

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