

SFB 35 Colloquia in Membrane Transport

Venue: Medical University Vienna, Center for Physiology and Pharmacology,
Institute of Pharmacology, Waehringstrasse 13a, 1090 Vienna,

"Leseraum"

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Host: Gergely Szakács

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"A transporter, a nuclease and a metabolite: the trio orchestrating connective tissue calcification"

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Pyrophosphate (PPi) is a central metabolite in preventing calcification in soft tissues, a pathological condition accompanied with many diseases. The liver is the most important source of circulatory PPi, via a pathway depending on a transporter (ABCC6)-mediated ATP release and rapid conversion of released ATP into AMP and PPi within the liver vasculature by the nuclease, ENPP1. Inactivating mutations in the ABCC6 and ENPP1 result in rare hereditary calcification disorders pseudoxanthoma elasticum (PXE) and Generalized Arterial Calcification of Infancy (GACI), respectively. While in PXE plasma PPi concentration is reduced to 40% of normal, GACI patients have virtually no PPi in their blood, which explains the extreme severity of the later disease.

We have followed two different experimental strategies to normalize plasma PPi in animal models of the two disorders.

I. Most mutations in ABCC6 are missense, and many of these mutations preserve transport activity but cause intracellular retention. We have shown that the chemical chaperone 4-phenylbutyrate (4-PBA) promotes the maturation of ABCC6 mutants to the plasma membrane. In a humanized mouse model of PXE, we investigated whether 4-PBA treatments could rescue the calcification inhibition potential of selected disease-causing ABCC6 mutants. We used the dystrophic cardiac calcification (DCC) phenotype of *Abcc6*^{-/-} mice as an indicator of ABCC6 function to quantify the effect of 4-PBA on human ABCC6 mutants transiently expressed in the liver of these animals. 4-PBA administrations restored the physiological function of ABCC6 mutants resulting in calcification inhibition. This study identifies 4-PBA treatments as a promising strategy for allele-specific therapy of ABCC6-associated calcification disorders.

We have developed a monoclonal antibody recognizing extracellular epitope of ABCC6, thus providing a useful tool in screening for novel chemical chaperons.

II. It was always assumed that the bioavailability of orally administered PPi is zero,

therefore such a supplementary therapy is not possible. In contrast to this assumption, we detected increased PPI concentrations in the circulation of humans that ingesting pyrophosphate. In mouse models of PXE and GACI, PPI provided via the drinking water attenuated the ectopic calcification phenotype (skin, kidney and arteries). Strikingly, providing drinking water with 0.3 mM PPI to mice heterozygous for inactivating mutations in *Enpp1* exclusively during pregnancy, robustly inhibited ectopic calcification in their *Enpp1*^{-/-} offsprings.

Our work shows that orally administered PPI is readily absorbed in human and inhibits connective tissue calcification in mouse models of PXE and GACI. PPI. PPI is recognized as safe by FDA, therefore not only has great potential as an effective and low cost treatment for these currently intractable genetic disorders, but also in other conditions involving connective tissue calcification. Such conditions and our related preliminary observations will also be discussed.

References:

Pomozi et al, (2017) *J. Invest. Dermatol.* 137:595-602
Dedinszki et al, (2017) *EMBO Mol. Medicine* 9: 1463-70