CHARACTERIZATION OF NOVEL BARTTER SYNDROME MUTATIONS IN CLCNKB AND CHAPERONE EFFICACY OF CLC-KB LIGANDS TOWARDS A PERSONALIZED MEDICINE

Dalila Sahbani[1], Bice Strumbo[2], Silvana Tedeschi[2], Giuseppe Procino[3], giovanni Montini[4], Diana Conte[1], Paola Imbrici[1], Antonella Liantonio[1]

[1]Department of Pharmacy-Drug Sciences, University of Bari "Aldo Moro", Bari - Italy, [2]Laboratory of Medical Genetics, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan - Italy, [3]Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari "Aldo Moro", Bari - Italy, [4]Pediatric Nephrology, Dialysis, and Transplant Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, and Department of Clinical Sciences and Community Health, University of Milan, Milan - Italy

Introduction: Type III and IV Bartter syndromes (BS) are rare kidney tubulopathies caused by loss-of-function mutations in the CLCNKB and BSND genes coding for ClC-Kb chloride channels and accessory subunit barttin (Andrini et al., Am J Physiol Renal Physiol 2015). ClC-K channels are expressed in the Henle's loop, distal convoluted tubule and cortical collecting ducts of the kidney and contribute to chloride absorption and urine concentration (Jentsch and Pusch, Physiol Rev 2018). We identified two new mutations in CLCNKB, G167V and G289R, and a previously reported mutation, A242E, in Italian patients affected by BS. This latter mutation reduced ClC-Kb membrane expression and, consequently, chloride current levels (Cheng et al., J Physiol 2017). All the patients had hypokalemia and metabolic alkalosis, increased serum renin and aldosterone levels and were treated with a symptomatic therapy. The aim of this study was to functionally characterize the new BS mutations of ClC-Kb channel, G167V and G289R, in order to define the molecular mechanisms responsible for BS. In addition, we attempted to revert the functional defect caused by ClC-Kb mutations G167V and A242E through barttin overexpression. In parallel, we tested the hypothesis that selective ClC-Kb ligands, including niflumic acid, valsartan, indometacin and SRA-36, can act as pharmacological chaperones of the same mutant channels (Imbrici et al., BBA 2014; Br J Pharmacol 2017).

Materials and methods: ClC-Kb WT and mutant channels were co-expressed with barttin (1:1ratio and 1:3ratio) in HEK293cells and chloride currents recorded through the patch-clamp technique. Confocal microscopy was used to detect membrane expression for ClC-Kb WT and mutants. For the pharmacological experiments, transfected cells were incubated for 24h with ClC-Kb-targeting drugs or vehicle, and chloride currents were recorded after drug washout. ClC-Kb expressing cells were also incubated with two drugs that have poor selectivity against ClC-Kb channels to verify the specificity of the chaperone effect.

Results: G167V channels showed a drastic current reduction compared to WT, likely suggesting compromised expression of mutant channels on the plasma membrane and confirming a genotype-phenotype correlation. The same defects were confirmed for the A242E channels. Conversely, G289R channel was similar to WT raising the doubt that an additional mutation in another gene or other mechanisms could account for the clinical phenotype. Interestingly, increasing ClC-K/barttin ratio augmented G167V and A242E chloride current amplitudes towards WT levels. In addition, niflumic acid 100mM, valsartan 50mM, indomethacin 100mM and the benzofuran derivative SRA-365mM increased G167V and A242E chloride currents amplitudes towards WT levels. As expected, neither losartan 50mM nor carbamazepine 100mM were able to increase the current amplitude of G167V and A242E channels after 24h incubation.

Discussion and conclusions: These results suggest that the functional characterization of mutant channels is fundamental both for the genotype-phenotype correlation and to choose the best therapeutic strategy. In this frame, we provide a preclinical proof-of-concept that small ClC-Kb reversible ligands, able to favor ClC-Kb membrane transport, can recover the activity of expression-defective mutants associated with BS, opening the way for a personalized mechanism-based therapy for BS patients.