BARTTER'S SYNDROME AND KIDNEY CLC-K CHANNELS: A PHARMACOVIGILANCE-BASED DRUG DISCOVERY STRATEGY TO REPROFILE SARTANS AS NOVEL LIGANDS

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Human CLC-Ka and CLC-Kb channels are expressed, with their accessory subunit barttin (BSND), in the kidney where they contribute to chloride absorption and urine concentration (Kramer et al., 2008). Gain-of-function polymorphisms in CLCNKA and CLCKNB genes predispose to a rare form of salt sensitive hypertension. Loss-of-function mutations in CLCKNB and BSND genes, affecting either channel open probability or trafficking, cause type III and IV Bartter's syndromes (BS), rare diseases characterized by impairment of urinary concentration ability. The pathogenetic mechanisms underlying BS are under explored as only a few mutations have been characterized so far (Andrini et al., 2015). These CLC-K-associated disorders lack a personalized and specific therapy that is in most cases only symptomatic. Thus, the development of selective CLC-K ligands would be decisive to meet patients' therapeutic needs: CLC-K activators would be necessary for BS whereas CLC-K blockers would be required to treat hypertension. Of note, selective and reversible CLC-K inhibitors could be potential pharmacological chaperones, favoring the membrane translocation of trafficking-defective BS mutants. Through structure and ligand-based strategies, we previously defined the pharmacophoric structures to activate and block the channels and demonstrated that niflumic acid, a non steroid anti-inflammatory drug, is the only known opener of CLC-K channels (Liantonio et al., 2008). We also showed that benzofuran derivatives are powerful CLC-K blockers, able to reduce blood pressure in preclinical studies (Liantonio et al., 2016).

The aim of this study was to discover novel CLC-Ks ligands among commercial drugs, with prompt-to-use therapeutic potential against CLC-K associated diseases.

Here we used an innovative drug discovery strategy based on the analysis of Food and Drug Administration-Adverse Effects Reporting System database monitoring drug safety. We searched for marketed drugs inducing BS-like syndrome as side effect with the assumption that BS could result from the block of CLC-K channels. This hypothesis was then validated through patch clamp electrophysiology in vitro, by testing the ability of the selected BS-causing drugs to inhibit CLC-K channels expressed in HEK293 cells. Induced-fit docking simulations were used to further validate the experimental findings and to shed light on the molecular determinants of channel block.

From the analysis of pharmacovigilance registries we selected several drugs inducing BS-like syndrome in patients undergoing antihypertensive, immunosuppressive and anticoagulant therapy, that were suitable for in vitro screening. We discovered that Valsartan and Olmesartan, two angiotensin II receptor (AT1R) blockers, were able to selectively block CLC-Ka channels with an IC50 of 20microM. Moreover, structure-activity studies suggested that both the tetrazole ring and the carboxylic group of the sartan are required for CLC-K block and that residues N68 and K165 of the channel are pivotal for this interaction. In parallel, on the basis of a collaboration with

clinicians, we collected 20 new BS mutations in the Italian population and functional and pharmacological characterization of two of these is ongoing.

In conclusion, we reprofiled Valsartan and Olmesartan as CLC-K ligands. These drugs could provide new scaffold to develop selective CLC-K ligands, and could be potentially useful as chaperons to rescue folding-defective BS mutants. This pharmacovigilance-based strategy could represent an alternative approach to discover ligands of other ion channels among commercial drugs, ensuring rapid clinical application.

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