SEARCHING FOR NOVEL DRUGS IN MYOTONIA CONGENITA: EVALUATION OF ANTHRACENE-9-CARBOXYLIC ACID AND NIFLUMIC ACID BINDING SITES AND CHAPERONE ACTIVITY ON CLC-1 CHLORIDE CHANNEL MUTANTS.

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Myotonia congenita (MC) is an inherited muscle disease characterized by impaired muscle relaxation after voluntary contraction, resulting in muscle stiffness. It is caused by loss-of-function mutations in the skeletal muscle chloride channel ClC-1, affecting its biophysical properties or membrane expression. Currently no direct activators of ClC-1 are known and symptomatic treatment is based on the use of sodium channel blockers.

An ideal drug would be one able to directly open ClC-1 channel in case of gating-defective mutants, or one capable to increase surface expression in case of trafficking-defective MC mutants.

The final aim of this study is to develop drugs acting on ClC-1 channels as a new class of antimyotonic drugs. For this purpose, we used small molecules reversible ClC-1 blockers, namely anthracene-9-carboxylic acid (9-AC) and niflumic acid (NFA), to gain information regarding channel gating and binding sites in ClC-1 protein and to perform proof-of-concept studies to verify the ability of pharmacological chaperones to restore membrane expression of trafficking-defective MC mutants.

To define binding pockets, we performed docking studies on a homology model built upon the crystal structure of an eukaryotic ClC to identify residues that play a central role in 9-AC and NFA binding. Docking results were confirmed using patch-clamp analysis of specific ClC-1 mutants. To yield additional insight into 9-AC binding site, we took advantage of other five ClC-1 mutations recently identified in Italian families affected by myotonia congenita (Imbrici et al., 2015; Desaphy et al., 2013; Portaro et al., 2014) that fall within the pore region.

We demonstrated that 9-AC binds within the pore and that removing the positive charge of Lys231 or replacing the aromatic ring of Phe484 nullifies 9-AC interaction with ClC-1. We showed that residues located in helices D (Gly190, Leu198) and G (Gly270) may be crucial for 9-AC to reach its binding sites and block ClC-1 channels, directly or through conformational changes.

In addition we mapped for the first time three putative cavities on ClC-1 for NFA binding and we focused on the pocket that returned the highest docking score and includes residues Arg421, Thr550, Phe484 and Phe488. The sensitivity of mutant channels to NFA assayed by patch-clamp suggest that the binding pocket of NFA is centred on Arg421 and Thr550 residues.

To investigate whether 9-AC and NFA may work as pharmacological chaperones, we selected a myotonic ClC-1 mutation (A531V) that shows a reduction of channel surface expression due to increased protein degradation (Desaphy et al., 2013; Chen et al., 2015). Incubation of transfected

cells for 24 hours with 30 µM 9-AC and 50 µM NFA increased both instantaneous and steady-state A531V chloride currents compared with control conditions, maintaining voltage-dependence similar to WT.

In conclusion, our results confirm that 9-AC and NFA are specific modulators of the skeletal muscle ClC-1 channel and are able to interfere with channel gating. The clarification of the binding sites of these compounds may allow a rational design of new drugs useful in the treatment of ClC-1-associated diseases. Moreover, this study provides a proof of concept that pharmacological chaperones may prove useful to restore trafficking-defective ClC-1 mutants and sarcolemma chloride conductance in myotonic patients.

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