PHARMACOLOGICAL CHAPERONE TO TREAT MYOTONIA CONGENITA CAUSED BY TRAFFICKING-DEFECTIVE CLC-1CHLORIDE CHANNEL MUTANTS: PROOF-OF-CONCEPT WITH NIFLUMIC ACID

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Introduction: Myotonia congenita (MC) is a rare disease characterized by sarcolemma over-excitability inducing skeletal muscle stiffness. It is the most common skeletal muscle channelopathy, caused by loss-of-function mutations in the ClC-1chloride channel. Therapy is symptomatic, based on the sodium-channel blocker mexiletine, with efficiency and safety concerns. No selective ClC-1activator is available. The MC mutations reduce chloride currents by altering the gating of ClC-1channel (gating defect) or by decreasing its cell surface expression (trafficking defect). One interesting strategy to restore protein surface expression is based on the use of pharmacological chaperones, molecules able to bind and stabilize misfolded mutated proteins. To test this hypothesis in MC, we use Niflumic Acid (NFA), a well-known reversible inhibitor of ClC-1,that likely binds to a hydrophobic pocket near the pore (Altamura et al., Br J Pharmacol 2018). Chaperone activity of NFA was tested on three myotonic hClC-1mutations that show a drastic reduction of chloride currents, due to an impaired channel surface expression. The selected MC mutations are p.A531V located in the helix O, p.V947E sited in the intracellular C-terminal, and the novel p.G411C between helix K and L. (Desaphy et al., Exp Neurol 2013; Altamura et al., Hum Mut 2018).

Methods: The recombinant wild-type (WT) human ClC-1channel and the MC mutants obtained using site-directed mutagenesis were expressed in HEK293cells. Whole-cell chloride currents were recorded in transfected cells with the patch-clamp technique, in control condition and after 24h incubation with NFA. Membrane biotinylation assays and fluorescent cell imaging of YFP-tagged ClC-1mutants were used to detect and quantify ClC-1membrane surface expression before and after NFA incubation.

Results: Expression of A531V and V947E channel mutants yield chloride currents similar to WT channels but with reduced amplitude, whereas no chloride current were detected in G411C-transfected cells. Fluorescent cell imaging of YFP-tagged G411C mutants confirmed that G411C channels are retained within the cell. Acute exposition to NFA inhibited WT chloride currents at -90 mV with an IC_{50} of 97μM. Acute effects of NFA on A531V and V947E currents were reduced. Incubation of transfected cells for 24hours with 50 μM NFA enhanced A531V and V947E chloride current density more than two fold (chloride currents were recorded after NFA washout), thereby restoring chloride current amplitude similar to WT. In contrast, NFA incubation did not allow to record chloride currents in cells transfected with G411C, suggesting lack of cell surface expression or complete loss of channel function. Biotinylation assays merely confirmed the results obtained with patch-clamp.

Discussion and conclusion: The results provide a proof-of-concept for the usefulness of pharmacological chaperones in restoring some trafficking-defective chloride channel mutants. It is worth to note that the concentration of NFA used here (50 μM) is within the range of therapeutic blood concentrations in humans (7-124μM). Because of the favourable safety profile of NFA, our study may easily open the way for confirmatory human pilot studies aimed at verifying the antimyotonic activity of NFA in selected patients carrying defective-deficient ClC-1channel mutations. A limitation of NFA may be its acute inhibitory activity on ClC-1channels. Yet, this compound could be the starting point for the development of more specific derivatives, paving the way toward personalized medicine for MC. (supported by Telethon-Italy GGP14096).