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P4.45
Evaluation of the dual action of new derivatives of mexiletine as use-dependent sodium channel blockers and antioxidant: potential therapeutic application in neuromuscular disorders

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Mexiletine (Mex), a voltage-gated Na+ channel blocker with anti-arrhythmic and anti-myotoxic effects, has been recently withdrawn from the market, pushing the search of new drugs for muscle disorders. A pyrrole derivative of Mex and its in vivo nitroxide metabolite protects against reperfusion injury, presumably due to a combination of antioxidant and membrane stabilizing mechanisms (Tomney et al., Free Radic Biol Med, 1997). This dual action is of interest for further enlarging the therapeutic potential of Mex-like compounds in different myopathies. Thus, we synthesized new compounds with a pyrrole ring, the reactive oxygen species (ROS) scavenging moiety, on the amino group of both Mex (VM11) and of its potent use-dependent isopropyl derivative (CI16). The new analogues were evaluated for their ability to block native skeletal muscle sodium channel (Nav1.4) by voltage-clamp recordings and to protect C2C12 cells from H2O2 cytotoxicity. The tonic (TB) and use-dependent (BUD) block of peak sodium currents (INa), produced by each drug were evaluated by using depolarizing pulses at low and high stimulation frequencies, respectively. VM11 and CI16 were 3 and 6-fold more potent than Mex in producing a TB of INa with IC50 values of 23.4 ± 0.9 μM and 12.6 ± 0.2 μM, respectively. Importantly, these compounds are among the most potent use-dependent Mex analogues described so far. In fact, CI16 showed a 20-fold increase of potency during high-frequency stimulation. Preliminary experiments show that Mex had a cytoprotective effect at the concentration as high as 1 mM, while CI16 produced a significant, although modest, reduction of H2O2-induced cytotoxicity at concentrations close to the IC50 value for TB of Nav1.4. The modest antioxidant effect in vitro suggests an important anti-oxidant action by the in vivo nitroxide metabolite, providing the basis for further in vivo/ex vivo studies. (Supported by the "Association Française contre les Myopathies").

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P4.47
Hereditary inclusion body myopathy therapy: Assessment of an adeno associated virus AAV2/8 based GNE gene delivery system
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Hereditary inclusion body myopathy (HIBM) is a unique neuromuscular disorder characterized by adult onset and slowly progressive distal and proximal muscle and a typical histology including rimmed vacuoles. The disease is caused by recessive mutations in the UDP-N-acetylgalactosamine 2-epimerase/N-acetylmannosamine kinase gene (GNE), encoding the key enzyme in the biosynthetic pathway of sialic acid. In an attempt to develop a possible treatment for HIBM patients, we have established a platform for gene therapy trials based on adeno associated virus (AAV 8) vectors harboring the wild type GNE gene. These viral constructs proved to be efficient in the infection of cultured human muscle cells derived form HIBM biopsies. C57Bl6 mice were injected either IM (gastrocnemius or quadriceps) or IV (tail vein) with wtGNE engineered AAV2/8 virus. No mice presented any physical or behavioral change. Tissues were analyzed for histology, inflammation and GNE expression 35 days post IM injection. hGNE was expressed mostly in the injected muscle, liver, kidney, heart, and spleen. Measurement of mRNA with specific human versus mouse probes revealed an increase in virus-derived human GNE expression. More importantly, DMRV/hIBM mice injected with AAV2/8-wt hGNE at 47 weeks of age showed a significant improvement in survival, motor performance, muscle size and contractile properties, as compared to those mice injected with AAV2/8-luciferase. These results establish a proof of principle in using AAV-mediated gene therapy for DMRV/hIBM.

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OTHER DISORDERS: POSTER PRESENTATIONS

P4.46
Expression of human GNE through adeno-associated virus mediated therapy delays progression of myopathy in the DMRV/hIBM mouse model

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Distal myopathy with rimmed vacuoles (DMRV) or hIBM is an early adult, moderately progressive myopathy due to mutations in the GNE. This gene encodes a bifunctional enzyme critical to synthesis of sialic acid. DMRV/hIBM so far has been shown to be a disorder due to reduced sialylation of certain glycoconjugates in tissues including the muscle, as replenishment of sialic acid in the murine model has prevented the onset of a muscle phenotype. The caveat in sialic acid supplement therapy, however, lies on the pharmacokinetic properties of sialic acid, as it is rapidly excreted after oral administration, raising the need to explore other modalities. This paper thus aims to demonstrate the utility of gene therapy in ameliorating the muscle phenotype of the DMRV/hIBM mouse model. In this study, we generated AAV expressing the human wt-GNE cDNA and enhanced green fluorescent protein (eGFP) under the transcriptional control of cytomolgavirus promoter in AAV 2/8 capsids. AAV 2/8 that carry either human wt GNE or luciferase (in control virus) were injected intravenously to adult and symptomatic DMRV/hIBM mice. Unaffected littersmates were also injected for control. At 10 weeks after injection, eGFP expression was seen in remarkable number of cells in the skeletal muscle, liver, kidney, heart, and spleen. Measurement of mRNA with specific human versus mouse probes revealed an increase in virus-derived human GNE expression. More importantly, DMRV/hIBM mice injected with AAV2/8-wt hGNE at 47 weeks of age showed a significant improvement in survival, motor performance, muscle size and contractile properties, as compared to those mice injected with AAV2/8-luciferase. These results establish a proof of principle in using AAV-mediated gene therapy for DMRV/hIBM.

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