DEVELOPMENT OF NEW BENZOTHIAZOLAMINES TO ENHANCE USE-DEPENDENT INHIBITION OF VOLTAGE-GATED SODIUM CHANNELS

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Introduction: Two drugs containing a benzothiazole scaffold, such as riluzole and lubeluzole, are potent inhibitors of skeletal muscle voltage-gated sodium channels hNav1.4(Desaphy et al., Mol Pharmacol 2013). Riluzole is indicated for amyotrophic lateral sclerosis, while lubeluzole has been tested in humans for neuroprotection in stroke. Both drugs also showed potent antimyotonic activity *in vivo* in an animal model of myotonia congenita, a rare disease characterized by skeletal muscle overexcitability and stiffness (Desaphy et al., Exp Neurol 2014). The sodium channel blocker mexiletine is the first-line drug for myotonia, but some patients show sub-optimal response or side effects. Here, we performed chemical maneuvers on riluzole and lubeluzole with the aim of exalting potency and use-dependent block of sodium channels.

Methods: Riluzole and marketed analogs were purchased. Lubeluzole and its derivative, as well as newly-designed riluzole derivatives, were synthesized in our medicinal chemistry unit. The compounds were tested on sodium currents recorded with patch-clamp in HEK293cells transfected with hNav1.4. Riluzole and some derivatives were tested in vitro on rat muscle fiber excitability using two-intracellular microelectrode current-clamp technique. Lubeluzole and its derivatives were tested *in vivo* in the rat model of myotonia.

Results: One concern of lubeluzole is its ability to induce long QT. We synthesized LUB1, a new derivative with reduced lipophilia, by introducing hydroxyl groups in the two outermost aromatic cycles. Indeed, an increased lipophilia is thought to favor inhibition of cardiac hERG channels leading to QT interval lengthening. We previously showed that hydroxyl substitution in meta position of mexiletine aryl ring does not impair I_{Na} block (Desaphy et al., Front Pharmacol 2012), while reducing the potency on hERG channels (Gualdani et al, Pharmacol Res Perspect 2015). Compared to lubeluzole, LUB-1was slightly less potent but more use-dependent in blocking sodium currents. The IC_{50} of LUB-1for sodium current inhibition was 80 μM at 0.1Hz and 4μM at 10 Hz stimulation frequency (holding potential was -120 mV). In the myotonic rat, lubeluzole exerted antimyotonic activity with an ED_{50} of ~0.1mg/kg. Unfortunately, LUB-1, tested at 1mg/kg, induced unexpected side effects, leading to interruption of *in vivo* experiments. We tested 19riluzole derivatives on hNav1.4channels. The deletion of the trifluoromethoxy group or its substitution by electron donators greatly impaired I_{Na} inhibition, whereas substitution by bulky/lipophilic groups had little consequence. Because riluzole and marketed derivatives are not use-dependent, we designed analogues with a more basic protonable amine group in position 2. Thus, introduction of a piperazine substituent (compound RIL-10) introduced significant use-dependent behavior, without altering potency compared to riluzole. Interestingly, RIL-10 was able to inhibit abnormal action potential firing induced by 9-anthracene carboxylic acid in rat myofibers, a model of myotonic sarcolemma over-excitability.

Discussion and conclusion: We obtained riluzole and lubeluzole derivatives with enhanced use-dependent behavior on hNav1.4sodium channels. Although LUB-1may have a reduced activity on hERG channels compared to lubeluzole, the derivative exerted unexpected side effects *in vivo*, due to either off-target activity or pharmacokinetic differences. Riluzole appears as a well optimized compound, since any of its derivatives showed increased potency on sodium currents. However, the possibility to increase use-dependency, as with RIL-10, opens the way to obtain safer drugs. These compounds will serve as leads to design compounds with major selectivity toward over-excited tissues, which may prove useful in myotonia (grant #19027supported by Association Française contre les Myopathies).