IN VITRO EFFECTS OF NOVEL CHOLINESTERASE INHIBITORS ON ENERGY METABOLISM

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Introduction

The trends of novel therapeutics for Alzheimer's disease (AD) treatment are focused on multitarget-directed ligands (MTDLs). MTDLs combine cholinesterase inhibition with additional biological properties such as antioxidant properties to positively affect neuronal energy metabolism and mitochondrial function. We examined the *in vitro* effects of 10 novel MTDLs on mitochondrial functions Derivatives of 7-methoxytacrine (7-MEOTA) were tested (Fig. 1):

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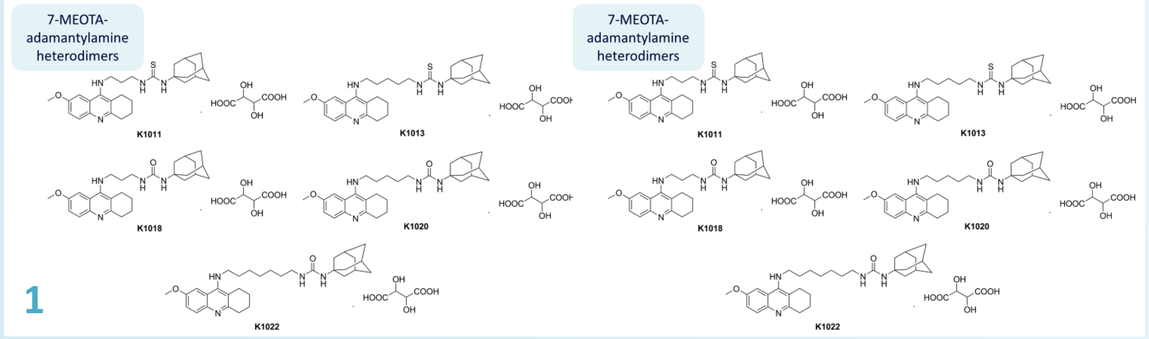

Methods

The drug-induced effects of novel MTDLs were measured in pig brain mitochondria. Activity of mitochondrial enzymes were measured spectrophotometrically. Mitochondrial respiratory rate was determined using high-resolution oxygraph. Effects on both MAO-A and MAO-B isoforms were examined radiochemically, using radiolabeled substrates (serotonine for MAO-A, phenylethylamine for MAO-B). Statistical analyses were performed using the STATISTICA data analysis software system.

Results – MAO activity

The novel compounds were full or partial inhibitors of both the MAO-A and MAO-B isoforms. MAO-A activity was fully inhibited by K1018, K1065, and 7-MEOTA, other compounds were partial MAO-A inhibitors. Clorgyline, as a selective MAO-A inhibitor, is included for comparison (Fig. 2).

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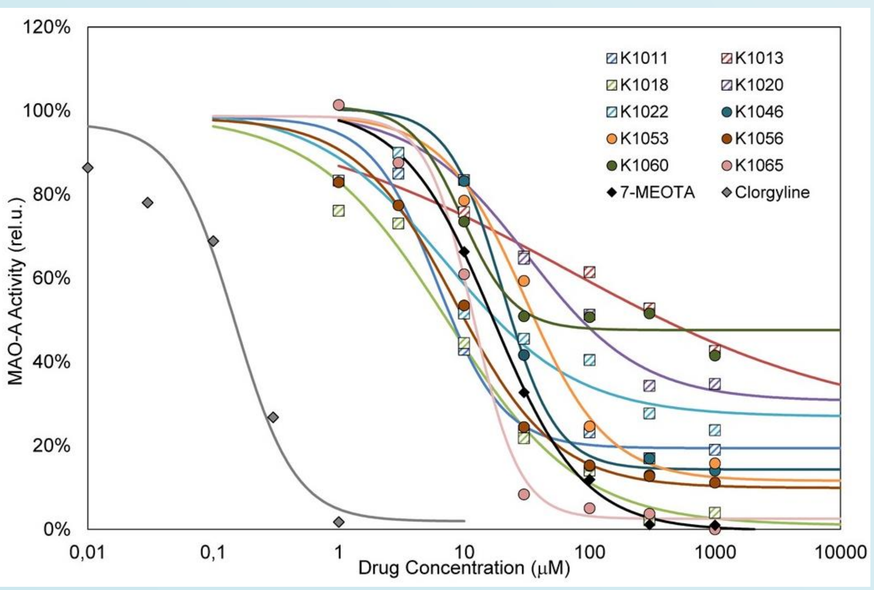

Full MAO-B inhibition was observed for K1053 and K1065, which were identified as suitable candidates in further trials. Partial inhibition of MAO-B by other tested substances K1060, K1056, K1046, K1020, K1018, and K1013 also indicates their suitability for use due to their potential neuroprotective effects. Clorgyline, as a selective MAO-A inhibitor, is included for comparison (Fig. 3).

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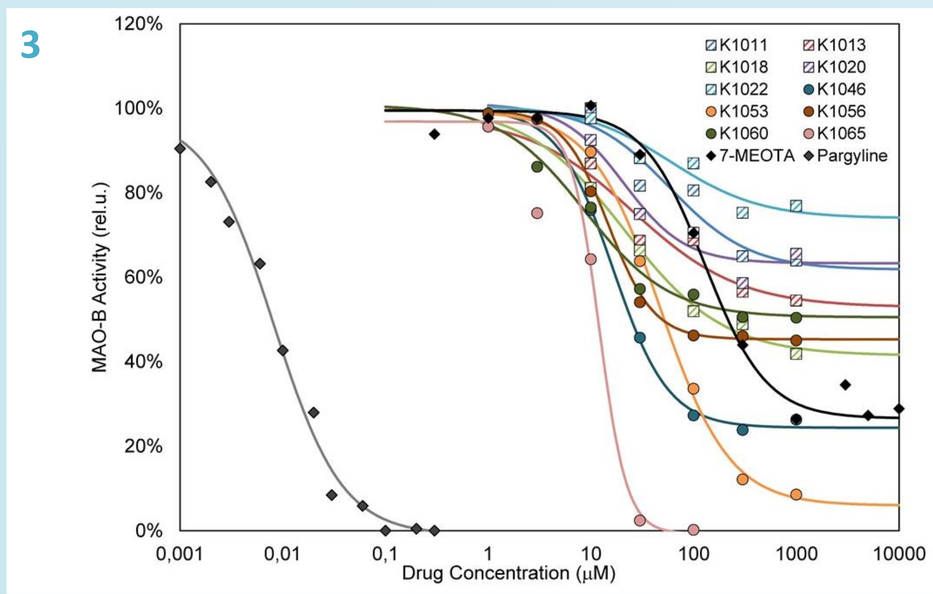

Results – mitochondrial respiration

Most of the tested drugs inhibited complex I-linked respiration (malate, pyruvate, ADP used as substrates). Except for K1011 and K1018, all the drugs could be considered selective inhibitors of complex I-linked respiration. The most potent inhibitor of complex I-linked respiration was K1018, and the weakest inhibitors were K1046, K1056 and K1060. The drug-induced inhibition of complex I-linked respiration is depicted in Fig. 4. Complex II-linked respiration (succinate, ADP as substrates, rotenone to block complex I activity) was strongly inhibited by K1018; full inhibition was caused by K1011 and K1013. Partial inhibition was observed after incubation with K1020 and K1065, and other newly developed ChE inhibitors did not inhibit respiration mediated by complex II substrates. Drug-induced complex II-linked inhibition is depicted in Fig. 5.

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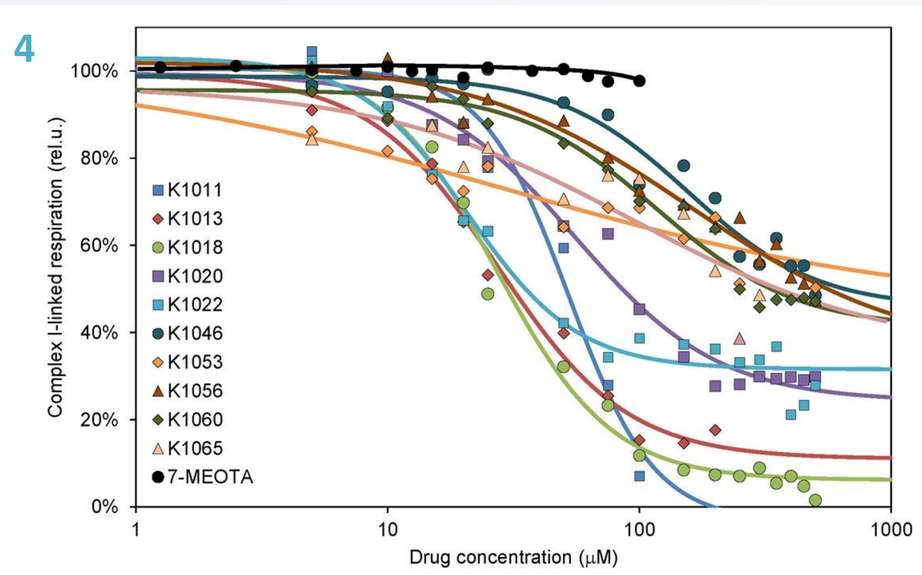

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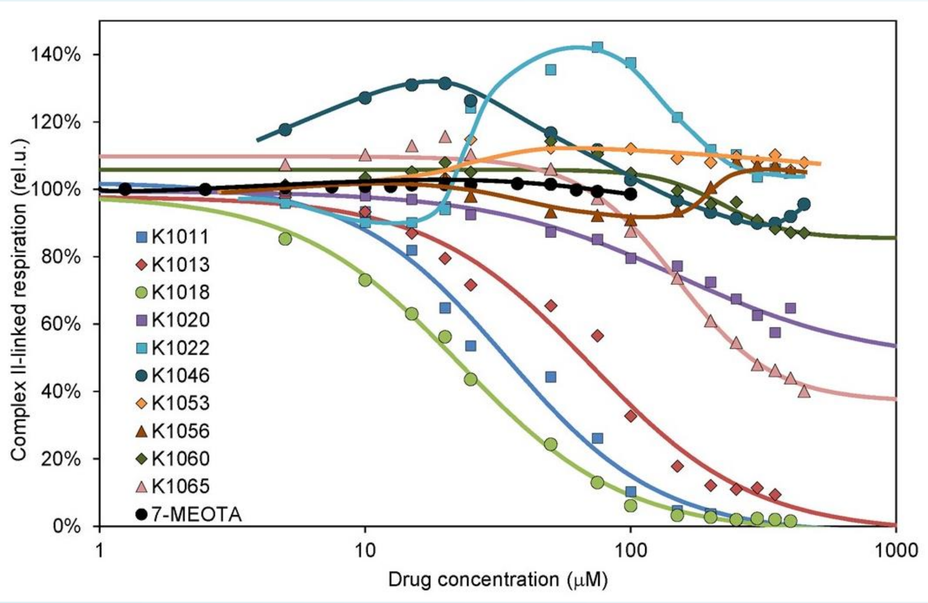

Discussion

Many of tested drugs have been shown to induce inhibition of complex I and complex I-linked respiration. It can be presumed that drug-induced inhibition of the mitochondrial respiratory rate is associated with impaired OXPHOS function and energy metabolism in neurons. Oxidative stress has been known to play a vital role in the pathophysiology of AD, and inhibition of complex I can affect ROS production. Generally, the inhibition of MAO-A is therapeutically exploited for its antidepressant effects, while MAO-B inhibition is responsible for a decrease in neurotoxic compound production, which enhances neuronal survival and provides neuroprotective effects. Both MAO-A and MAO-B inhibition slow down the metabolism rate of monoamines and lead to decreased ROS production of potential drugs developed for AD treatment. The inhibition of MAO-B activity is one of the mechanisms of many multitarget-directed drugs; MAO-B inhibition could be linked to the neuroprotective effects of the candidate AD molecules.

Conclusions

Tacrine/7-MEOTA/6-chlorotacrine-trolox heterodimers, namely, K1046, K1053, and K1060, were found to have balanced inhibitory effects on mitochondrial respiration, a low inhibitory effect on complex I and complex II/III, no effect on complex IV activity, and a sufficient inhibitory effect on MAO-B activity. Therefore, these substances seem to be the most suitable molecules for other in vivo studies. MAO-B inhibition could be link to neuroprotective effects of these candidate molecules for AD treatment.

References:

1. Hroudova, J. & Fisar, Z. (2010) Neuro-Endocrinology letters, 31, 336-342.
2. Korabecny, J., Musilek, K., Holas, et al. (2010) Bioorganic & medicinal chemistry letters, 20, 6093-6095.

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