Commentary

**In vitro geroscience. Screening anti-aging drug combinations for neurodegenerative diseases.**

Soroosh Fatemi1, Joo Young park1, Warren Ladiges1

1Department of Comparative Medicine, School of Medicine, University of Washington, Seattle, WA

**Abstract**

Geroscience is based on the concept that therapeutic approaches that work for aging will work for age-related diseases including neurodegenerative conditions such as Alzheimer’s disease (AD). Single drugs have been ineffective in treating AD, so it seems reasonable to consider using multiple drugs in combination (cocktails) for a more effective treatment approach. However, initial screening of drug cocktails in animal models is costly and time consuming. The SH-SY5Y human neuroblastoma cell line exhibits neuronal cell properties and can be stressed with chemicals or transfected with AAV Aβ and/or pTau vectors for an in vitro model of neuro-toxicity. Drug cocktails can then be easily screened for intervention effectiveness compared to each individual drug in the cocktail.

**Key word**. Drug cocktails, Anti-aging drugs, Geroscience, Neuroblastoma cell line, Alzheimer’s disease

**Correspondence.** Warren Ladiges, wladiges@uw.edu

Alzheimer’s disease (AD) is a common neurodegenerative condition that has, as yet, not successfully responded to single drug therapeutics. The geroscience concept assumes that therapeutic approaches that work for aging will work for age-related diseases including AD. Therefore, rather than focus on testing a single drug, it seems reasonable to consider using multiple drugs in combination (a cocktail) for a more effective treatment strategy. However, testing drug cocktails is costly and time consuming in animal models.

A simple and inexpensive system is needed. In this regard, Mairuae et al [1] recently published an article describing the use of a human neuroblastoma cell line to test the ability of a combination of mulberry fruit and leaf extracts to prevent hydrogen peroxide-induced cytotoxicity. They showed that the protective effect was most pronounced with the extract combination compared to each individual extract, suggesting an interaction between the two extracts and that the extract combo hit a wider path of cellular targets.

Their observation reinforces the critical importance of using drug combinations to treat AD. However, this type of approach has not received the attention it deserves mainly because there is still a mystery as to what causes AD, and in fact there most likely are multiple causes. The mainstream thinking is that using drug combinations to treat AD is a shot gun approach and not scientifically sound. This is counterintuitive to the geroscience concept based on the underlying principle that if a drug cocktail is effective in delaying aging, then it will be effective in delaying dementia and neuropathology associated with AD. Our lab has recently shown that a combination of rapamycin, acarbose, and phenylbutyrate was effective in enhancing resilience to aging in C57BL/6 and HET3 mice [2]. We also showed that the drug combination delayed the onset of age-related cognitive impairment [3], suggesting it would be a promising combination to test for delaying or preventing AD.

Part of the reluctance to embrace drug combinations for treating AD might be the high cost and intense effort to conduct such studies in animal models. An in vitro cell culture system would be ideal to screen drug combinations in an inexpensive and timely manner, as described by Mairuae et al [1]. They used the SH-SY5Y human neuroblastoma cell line, which is of neuronal origin and exhibits neuronal cell properties including cytotoxicity influence. In order to use this cell line to screen drug combinations for AD, we have done transfections with AAV vectors containing sequences for Aβ42 and pTau. Drugs that are soluble in aqueous solutions, such as peptides, are easily tested in such a model system. However, many insoluable drugs can be solubilized in solvents such as DMSO and still be tested under the right conditions. Since we are not looking for one specific target, there is no need to use a reporter system. Instead, we have developed a streamlined immunohistochemistry format with multiple neuropathology markers as readouts to measure drug cocktail efficacy compared to each individual drug.

In conclusion, using the neuroblastoma SH-SY5Y cell line is one example of a viable, robust and inexpensive in vitro system to screen anti-aging drug cocktails as effective therapeutics for neuronal cell-damage associated with neurodegenerative conditions such as AD. By testing different combinations of drugs that target a wider range of aging pathways, it will be possible to screen candidate drug cocktails quickly and efficiently, and justify the time and expense to do larger-scale animal studies.

**References**

1. Mairuae N, Palachai N, Noisa P. The neuroprotective effects of the combined extract of mulberry fruit and mulberry leaf against hydrogen peroxide-induced cytotoxicity in SH-SY5Y Cells. BMC Complement Med Ther. 2023 Apr 13;23(1):117. doi: 10.1186/s12906-023-03930-z. PMID: 37055744; PMCID: PMC10100183.

2. Jiang Z, Wang J, Imai D, Snider T, Klug J, Mangalindan R, Morton J, Zhu L, Salmon AB, Wezeman J, Hu J, Menon V, Marka N, Neidernhofer L, Ladiges W. Short term treatment with a cocktail of rapamycin, acarbose and phenylbutyrate delays aging phenotypes in mice. Sci Rep. 2022 May 4;12(1):7300. doi: 10.1038/s41598-022-11229-1. PMID: 35508491; PMCID: PMC9067553.

3. Jiang Z, He Q, Ladiges W. A cocktail of rapamycin, acarbose and phenylbutyrate prevents age-related cognitive decline in mice by altering aging pathways. bioRxiv (2022): 2022-09.