PERSPECTIVES

Using Plants as a Source of Potential Therapeutics for the Treatment of Alzheimer's Disease

Pamela A. Maher*

Salk Institute for Biological Studies, La Jolla, CA

Alzheimer's disease (AD) is the most common form of dementia with the numbers expected to increase dramatically as our society ages. There are no treatments to cure, prevent, or slow down the progression of the disease. Age is the single greatest risk factor for AD. However, to date, AD drug discovery efforts have generally not taken this fact into consideration. Multiple changes associated with brain aging, including neuroinflammation and oxidative stress, are important contributors to disease development and progression. Thus, due to the multifactorial nature of AD, the one target strategy to fight the disease needs to be replaced by a more general approach using pleiotropic compounds to deal with the complexity of the disease. In this perspectives piece, our alternative approach to AD drug development based on the biology of aging is described. Starting with plants or plant-derived natural products, we have used a battery of cell-based screening assays that reflect multiple, age-associated toxicity pathways to identify compounds that can target the aspects of aging that contribute to AD pathology. We have found that this combination of assays provides a replicable, cost- and time-effective screening approach that has to date yielded one compound in clinical trials for AD (NCT03838185) and several others that show significant promise.

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia. The main pathological characteristics are the accumulation of extracellular neuritic plaques containing amyloid beta (Aβ) peptide and intracellular neurofibrillary tangles containing tau [1]. The primary clinical symptom is a progressive loss of cognitive function which even-tually results in an inability to perform the activities of daily living [2,3]. The currently approved therapies only modestly and transiently reduce the clinical symptoms but fail to alter the course of disease progression [4,5]. Although there have been a large number of clinical trials in recent years with drug candidates designed to directly or indirectly reduce the amyloid plaque load, all of these trials have failed [6,7].

*To whom all correspondence should be addressed: Pamela A. Maher, Salk Institute for Biological Studies, 10010 N. Torrey Pines Rd., La Jolla, CA, 92037; Tel: 858-453-4100 x1932; Email: pmaher@salk.edu.

Old age is by far the greatest risk factor for AD [8,9]. However, AD drug discovery and AD clinical trials have largely focused on targets related to the familial form of AD (FAD) which only accounts for a small percentage (<5%) of the total cases [9]. The vast majority of AD cases are sporadic and may be quite distinct from the genetic form of the disease. There are a number of pathophysiological changes that occur in the aging brain that potentially contribute to the nerve cell damage and death that is characteristic of AD including increases in oxidative stress, alterations in energy metabolism, loss of neurotrophic support, alterations in protein processing leading to the accumulation of protein aggregates, dysfunction of the neurovascular system and immune system activation [10,11]. Given this wide range of age-related changes that occur in the brain, it seems unlikely that developing drugs that only modulate a single pathological change will prove effective at preventing the development and progression of AD. In addition, there is a strong possibility that the relative contributions of each of these changes will vary among individuals. Indeed, as Dale Bredesen [12] and others have pointed out, AD is pathologically heterogeneous and may require a "personalized" approach for treatment. Moreover, these age-associated changes in the brain are modulated by lifestyle as well as environmental and genetic risk factors. Thus, it is likely it will be necessary to use therapies directed against different targets in order to effectively prevent these multiple age-related changes to the brain. One approach now used in cancer therapeutics involves combinations of drugs. However, this approach is subject to a number of pharmacokinetic and bioavailability challenges because drugs for central nervous system (CNS) diseases need to get across the blood brain barrier as well as the potential for long-term adverse drug-drug interactions. A better approach is to identify small molecules that have multiple biological activities that can impact the multiplicity of age-associated pathophysiological changes in the brain that contribute to AD development and progression [13]. We have implemented this approach by using a novel battery of phenotypic screening assays that reflect multiple age-associated toxicity pathways as described below.

TOPICS

Approaches to Drug Discovery for Neurodegenerative Diseases

A mixture of molecular and structural biology, combinatorial chemistry, and high throughput screening has dominated the drug discovery process since the 1990s [14]. Although this approach provides a rapid process for the discovery of drug candidates with high selectivity and high affinity for a specific molecular target, it has not produced the successes that were initially expected. This is especially true for complicated indications such as neurodegenerative diseases, including AD. Prior to the development of this target-based drug discovery approach, new drugs were typically identified by an approach called phenotypic screening. Phenotypic screening involves evaluating compounds against observable characteristics or phenotypes in biological systems such as animals or cells. Although phenotypic screening is no longer favored by the pharmaceutical industry, it still continues to be more successful than target-based approaches for the identification of novel small molecule drugs [15]. It has been argued that this is because while phenotypic screening can incorporate the complexities of biological systems, target-based discovery cannot and also requires many more *a priori* assumptions that may not reflect the situation *in vivo* [10,15].

Many of the natural product-based, first in class drugs were originally discovered using the ultimate end user-humans [16,17]. However, this approach is no longer appropriate for drug discovery. Furthermore, while laboratory animals are often used for preclinical testing, their use for the initial screening of potential drug candidates is impractical due to cost and time constraints as well as ethically questionable. Thus, a better alternative is to create cell-based assays that reflect the molecular toxicity pathways that are relevant to age-associated neurodegeneration and select for further investigation potential drug candidates that work in multiple assays, not just one [10]. By using this approach, the cell-based assays should have disease relevance as well as reproducibility and reasonable throughput. Moreover, since arguments can be made against the relevance of any single cellular screening assay, based on the cell type or the nature of the toxic insult, phenotypic screening approaches for neurodegenerative diseases should combine multiple assays that address the different toxicities associated with the aging brain. Therefore, the assembly of our assay pipeline was based on the following considerations: 1) All assays are associated with pathology and molecular changes that are significantly altered in the normal aging brain relative to young brains; 2) The assays reflect conditions that are more robust in diseased brains relative to age-matched controls; 3) Different assays use distinct types of CNS cells in order to ensure that a candidate compound that is beneficial for one cell type does not have detrimental effects on the other; 4) Assays are conducted when the cell is physiologically stressed, either by a toxin or loss of trophic support. Ideal drug candidates should have no adverse effects on normal unstressed cells.

The assays that my colleagues and I have developed and characterized are described below and in Table 1. While the suitability of any one of these assays can be questioned on theoretical grounds, in combination, we

Table 1. Phenotypic Screening Assays.

Assay	Age-Related Toxicity
Oxytosis/ferroptosis	oxidative stress, lipid peroxidation
In vitro ischemia	energy loss
Microglial activation	inflammation
Trophic factor withdrawal	loss of trophic factors
PC12 neurites	loss of neuronal connections
Intracellular Aβ toxicity	intracellular protein aggregation

have found that they make reliable predictions about the neuroprotective effects of compounds *in vivo* that hold true across several dementia models, and are thus of significant practical use [10,13]. Moreover, the use of cell-based assays reduces the potential for animal toxicity and avoids false positive results attributed to pan assay interference compounds (PAINS) in single target assays [18,19]. In addition, we have found that this combination of assays provides a replicable, cost- and time-effective screening approach that has to date yielded one compound in clinical trials for AD (NCT03838185).

Phenotypic Screening Assays

1. Oxytosis/ferroptosis: This assay tests the ability of compounds to rescue cells from oxidative stress-induced cell death caused by glutathione (GSH) depletion [20]. A reduction in GSH is seen in the aging brain and is accelerated in many CNS diseases including AD [21]. Importantly, GSH loss in the brain is associated with impairments in cognitive function [21-23]. High levels of glutamate inhibit cystine uptake by the cystine/glutamate antiporter, system x_c, thereby leading to GSH depletion. Experimentally, oxytosis can be investigated in isolation from excitotoxicity in neuronal cell lines or immature primary cultured neurons devoid of NMDA receptors. The depletion of GSH from cells leads to reactive oxygen species production, lipoxygenase activation, lipid peroxidation, and calcium influx which initiates a form of regulated cell death with features of both apoptosis and necrosis [20]. All of these changes are implicated in the nerve cell damage and death seen in AD [24]. Oxytosis appears to be very similar, if not identical, to another recently described form of cell death called ferroptosis [25]. We have found that most, if not all, compounds that inhibit oxytosis also inhibit ferroptosis [26] while the ferroptosis inhibitor ferrostatin-1 can also inhibit oxytosis [27]. This is of relevance because oxytosis/ferroptosis has been implicated in a number of pathological processes including neurodegenerative diseases [20,28-30]. Because of the generality of the toxicity pathway in oxytosis and its mechanistic association with aging and age-associated neurodegenerative diseases such as AD, it is used

as our primary screen. In this assay, HT22 hippocampal neuronal cells or primary cortical neurons are treated with 5 mM glutamate for 24 hr and cell survival assayed by the MTT assay. In the absence of a neuroprotective compound ≥ 90% of the cells die under these conditions. The most effective compounds are protective when added at the same time as the glutamate or even several hours afterwards.

2. In vitro ischemia: A breakdown in neuronal energy production leading to ATP loss is associated with nerve cell damage and death in AD [31]. Therefore, maintenance of ATP levels is an important but overlooked therapeutic target. In order to induce ATP loss, we use the compound iodoacetic acid (IAA), a well-known, irreversible inhibitor of the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase [32], in combination with the HT22 mouse hippocampal neuronal cell line. IAA, which has been used in a number of other studies to induce *in vitro* ischemia [33-37] causes a rapid loss of ATP [32,38]. We have shown that this assay can identify compounds which can maintain ATP levels in the presence of stress [38] and has been used as a primary screen to identify novel and highly potent derivatives of the flavonoid fisetin [39]. In this assay, HT22 hippocampal neuronal cells are treated with 20 μM IAA for 2 hr which results in 90-95% cell death 20 hr later as determined by the MTT assay. The compounds are included during the IAA treatment and in the fresh medium added after the 2 hr IAA treatment.

3. Inhibition of microglial activation: Inflammation is a major feature of AD as well as essentially all other neurological disorders (for reviews see [40,41-43]). Microglia are the resident immune cell population of the CNS, comprising 5-10% of the total cell population (for reviews see [44-46]). Their presence can have both beneficial and detrimental effects on the brain. Classically activated microglia are implicated in the pathogenesis of a variety of neurological disorders including AD. These microglia produce a wide array of pro-inflammatory and cytotoxic factors including cytokines, free radicals, excitatory neurotransmitters and eicosanoids that may work in concert to promote neurodegeneration. In the context of the AD brain, there are thought to be multiple stimuli that

generate an inflammatory response in the microglia including Aβ [43]. Thus, inhibiting the generation of classically activated microglia is another important therapeutic target for AD. In this assay, the ability of a compound to inhibit the classical activation of the mouse microglial cell lines BV2 or N9 by bacterial lipopolysaccharide (LPS) is tested using the increased production of NO as a primary read-out [39,47].

4. Trophic Factor Withdrawal: As the brain ages, there is a loss of the trophic factors that maintain neuronal integrity [48,49]. A simple assay to identify compounds that protect from the loss of trophic factors that promote nerve cell survival involves plating rat embryonic cortical neurons at low density in standard tissue culture medium. Freshly plated cells die within 24 hr, while if the cells are plated in the presence of both fibroblast growth factor and brain-derived neurotrophic factor they survive [50]. Importantly, in the presence of compounds that can substitute for the absence of survival-promoting trophic factors, the cells will also survive.

5. PC12 Differentiation: Connections between nerve cells are also altered in AD. Thus, compounds that can promote the regeneration of these connections might be of particular benefit, thereby promoting the recovery of higher neuronal function. As a model for this property, we use neurite outgrowth in PC12 cells, a well-studied model system of neuronal differentiation. In response to neurotrophic factors such as nerve growth factor, PC12 cells undergo a series of physiological changes culminating in a phenotype resembling that of sympathetic neurons (for review see [51]). These changes are the result of the activation of a coordinated series of signaling pathways and include the cessation of cell division, the expression of genes encoding nerve cell-specific proteins and the extension of neuritic processes. In this assay, PC12 cells are treated with compounds in N2 medium and neurite outgrowth scored after 24 hr as described [52].

6. Intracellular amyloid toxicity: Many now consider the accumulation of intracellular Aβ as being a primary toxic event in AD [53-56]. The human nerve cell line MC65 conditionally expresses the C99 fragment of the amyloid precursor protein (APP) leading to the accumulation of intracellular Aβ. The MC65 cells are routinely grown in the presence of tetracycline and, following its removal, the expression of C99 is induced and the cells die within 4 days because of the accumulation of intracellular, toxic protein aggregates [57,58] which is associated with a pro-inflammatory response [59]. Cell death is not caused by the secretion of Aβ and extracellular toxicity, nor by secreted toxins [57]. Death is inhibited by γ-secretase inhibitors [60]. Cell death is easily measured by the MTT assay as there is complete cell lysis.

Together, this combination of phenotypic screening assays enables the identification of potent, disease-modifying compounds for preclinical testing in animal models of neurodegenerative diseases. Importantly, by means of these assays, we have successfully identified compounds with distinct targets that show beneficial therapeutic efficacy in *in vivo* models of AD [13,58,61-63]. Moreover, one of these compounds, the curcumin derivative J147, is in a phase 1 clinical trial for AD (NCT03838185) and another, the fisetin derivative CMS121 [13,39,63], is in IND-enabling studies for the treatment of AD. However, as noted earlier, AD is a heterogeneous disease so even if multi-target drugs are identified, it is likely that multiple drugs will be needed to successfully treat all of the patients with the disease. In addition, most drug candidates do not make it past clinical trials into the clinic so it is essential to have multiple candidates against distinct drug targets in the pipeline. Thus, we believe that our phenotypic screening paradigm should be very useful for identifying additional, new drug candidates for the treatment of AD and other age-related neurodegenerative diseases. With these ideas in mind, what should be screened?

WHY START WITH PLANTS?

One of the best sources of multi-target compounds are plants, the original pharmacopeia. Records describing the use of plants for medicinal purposes date back to 2600-2900 BC [64]. Even today, ~25% of all prescribed drugs are thought to be derived from plants [16]. Plants synthesize a huge array of compounds called secondary metabolites that are not required for plant development, growth, or reproduction but are needed for survival. Surprisingly, these compounds are derived from a limited number of basic chemical scaffolds which are modified by specific types of chemical substitutions. It has been suggested [16,65] that these compounds, as well as receptors, enzymes, and regulatory proteins, originated from a relatively small number of parental molecules which may have co-evolved to interact with one another. Although their biological functions and structures have since diverged, structural features shared from their common past may be the reason that they interact with medically relevant targets. Since plants have been used for millennia for medicinal purposes, one good starting point for compound selection is the literature on traditional medicine [17,64]. Traditional Chinese medicine in particular provides a very rich source of information about plants used to treat a variety of diseases including those involving the brain [66,67]. As described below, we have taken several different approaches to selecting the compounds or plants that we have screened. With all of these approaches, we were able to identify interesting compounds that show promise for the treatment of AD and are continuing to identify more. Three examples are described below.

Examples from our Lab

Example #1: We originally identified the flavonoid fisetin (3,7,3',4' tetrahydroxyflavone) in our screening assay for compounds that can prevent oxytosis/ferroptosis [68]. Of the ~30 flavonoids and related polyphenols tested in this study only two, fisetin and quercetin, were able to maintain GSH levels in the presence of oxidative stress, indicating that this is not a common property of flavonoids and related polyphenols. Further screening with many additional flavonoids and related polyphenols have confirmed this observation. Additional studies showed that fisetin also possessed neurotrophic activity, promoting the differentiation of PC12 cells via activation of the Ras-ERK cascade [52]. Again, this was a property that distinguished fisetin from almost all of the other ~30 flavonoids tested. Together, these observations suggested that fisetin had multiple properties that might make it useful for the treatment of AD. Further studies have demonstrated that fisetin can protect nerve cells from multiple toxic insults including Aβ toxicity, ischemia, and hyperglycemia. It has both direct antioxidant activity and maintains the levels of GSH under conditions of stress by inducing the transcription factors, Nrf2 and ATF4 [69]. Fisetin is also able to maintain ATP levels under ischemic conditions [38]. Moreover, fisetin was shown to facilitate long term potentiation (LTP) in hippocampal slices via modulation of ERK and CREB phosphorylation and oral administration of fisetin promoted learning and memory in mice using the object recognition test [70]. Fisetin is also effective in two different models of stroke [38,71]. Using three different models of Huntington's disease (mutant huntingtin-expressing PC12 cells, mutant huntingtin-expressing *Drosophila*, and the R6/2 mouse) we found that fisetin was able to reduce the impact of mutant huntingtin in each of these disease models [72]. Most relevant to this article, fisetin prevents learning and memory deficits in both APPswe/PS1dE9 (huAPP/PS1) double transgenic AD mice [62] and rapidly aging SAMP8 mice [73]. Work from other laboratories has confirmed and extended these studies both in additional preclinical models of AD [74] as well as preclinical models of Parkinson's disease [75] and amyotrophic lateral sclerosis [74]. Moreover, two recent studies showed that fisetin also has anti-aging properties [73,76] consistent with the idea that aging and neurodegenerative diseases are tightly linked.

However, fisetin's relatively high EC_{50} in cell based assays (2-5 μM) as well as its low lipophilicity (cLogP 1.24), high tPSA (107Å) and high number of hydrogen bond donors (HBD = 5) suggested that there was room for medicinal chemical improvement. Using structure-activity relationship (SAR)-driven iterative chemistry, we synthesized more than 160 derivatives of fisetin based on several different chemical scaffolds. We used a multi-tiered approach to screening that allowed us to identify fisetin derivatives with significantly enhanced neuroprotective activity in our *in vitro* neuroprotection assays while at the same time maintaining other key actions including anti-inflammatory activity [39]. While all of the fisetin derivatives had improved medicinal chemical properties more consistent with those of known CNS drugs, ~20 had greatly enhanced neuroprotective activity [39]. ADME and pharmacokinetic studies on the six most promising derivatives showed that several had peak brain levels following a single oral dose of 20 mg/kg that greatly exceeded their average EC_{50} in the *in vitro* neuroprotection assays as well as good oral bioavailability. Based on these and other results we selected the fisetin derivative CMS121 for further testing in preclinical mouse models of aging and AD [63].

Example #2: In a proof of principle study from the lab [77], extracts from five different species of plants – *Voacanga africana, Sacosperma paniculatum, Psychotria subobliqua, Psychotria principensis,* and *Tarenna nitiduloides* – from São Tomé and Príncipe were tested in our panel of phenotypic screening assays. Three of the species were selected on the basis of ethnopharmacological data while the other two had no reported ethnopharmacological relevance and were chosen as negative controls. Briefly, our workflow consisted of testing hydro-ethanolic extracts from the different candidate species and selecting the extract that performed best in all of the different assays for subsequent fractionation. The resultant individual fractions were tested in the oxytosis/ferroptosis assay and the predominant compound of the most active fraction was purified, structurally determined, and its efficacy tested in the complete phenotypic screening assay panel. An extract from the bark of *Voacanga africana* was more protective than any other plant extract in all of the assays (average EC_{50}s < 2.4 μg/ml). The HPLC fraction from the extract that was most protective contained the alkaloid voacamine as the predominant compound. To confirm this activity, voacamine was purified and tested and proved to be very effective in all five assays (average EC_{50}s < 3.4 μM).

Example #3: More recently we screened a commercial library of extracts from plants with known ethnopharmacological uses to identify additional potential new drug candidates for the treatment of AD [78]. All plant extracts were first tested in the oxytosis/ferroptosis assay described above. Extracts that were positive in this assay were then screened in the additional assays that are also described above. As already mentioned, these assays reflect multiple, age-associated neurotoxicity/survival pathways directly relevant to AD, such as increased oxidative stress and GSH depletion, reduced energy metabolism, accumulation of misfolded, aggregated proteins, loss of neurotrophic support and inflammation [10]. Using this approach, we identified an extract from Yerba santa
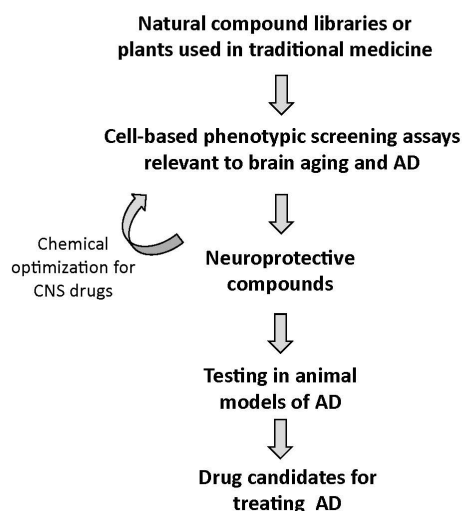
**Natural compound libraries or
plants used in traditional medicine**

⇩

**Cell-based phenotypic screening assays
relevant to brain aging and AD**

⇩

Chemical
optimization for   **Neuroprotective
CNS drugs   compounds**

⇩

**Testing in animal
models of AD**

⇩

**Drug candidates for
treating AD**

Figure 1. Schematic of screening workflow.

(*Eriodictyon californicum*), a shrub native to California, as being highly effective in all of our assays. Further analysis showed that the active component of this extract was the flavanone sterubin which had not been previously studied in the context of neuroprotection [78]. Additional experiments with pure sterubin confirmed that it was highly effective in all of our assays. Although sterubin is able to induce the anti-oxidant transcription factor Nrf2, this does not account for all of its neuroprotective effect because knockdown of Nrf2 using siRNA only partially reduces its ability to protect against oxytosis/ferroptosis and has no effect on its ability to protect cells against direct oxidants [78]. In addition, sterubin has strong anti-inflammatory effects as well as neurotrophic activity. All of these activities suggest that sterubin deserves further examination in the context of AD. Although sterubin has not yet been tested *in vivo*, its physicochemical properties are consistent with those of known CNS drugs suggesting that it has a good potential to cross the blood brain barrier and enter the brain [78].

CONCLUSIONS AND OUTLOOK

There are no effective treatments for age-dependent neurodegenerative conditions such as AD. To address this major public health crisis, one or more effective drugs are required. Since AD and related dementias are diseases that occur with age, aging must be incorporated into the drug candidate identification strategy. Thus, we have developed an age-related phenotypic screening platform that has already yielded compounds that are effective in pre-clinical models of AD and one of which is now in AD clinical trials (Figure 1). We believe that further

implementation of this platform with additional libraries of natural products not restricted to plants and including the vast amount of information available on traditional medical practices in China and elsewhere has the potential to identify new compounds for the treatment of AD and related neurodegenerative diseases. Although these compounds will need to be tested in animal models of AD, as we have done for fisetin [62,73], a major advantage of natural products is that in the US they generally don't need to undergo the rigorous and expensive Investigational New Drug approval process. Thus, if funding is available, they can move much more quickly into small scale clinical trials. With this approach, we believe it is possible to develop a battery of drugs to successfully treat AD within the time frame of that proposed by the National Alzheimer's Project Act (NAPA) whose goal is to find effective interventions to treat and prevent AD and related dementias by 2025.

REFERENCES

1. Goedert M, Spillantini MG. A century of Alzheimer's disease. Science. 2006 Nov;314(5800):777–81.
2. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA work group under the auspices of Department of Health and Humans Services Task Force on Alzheimer's disease. Neurology. 1984 Jul;34(7):939–44.
3. McKeith I, Cummings J. Behavioural changes and psychological symptoms in dementia disorders. Lancet Neurol. 2005 Nov;4(11):735–42.
4. Haas C. Strategies, development, and pitfalls of therapeutic options for Alzheimer's disease. J Alzheimers Dis.

2012;28(2):241–81.

5. Rafii MS, Aisen PS. Recent developments in Alzheimer's disease therapeutics. BMC Med. 2009 Feb;7:7.

6. Gold M. Phase II clinical trials of anti-amyloid β antibodies: when is enough, enough? Alzheimers Dement (N Y). 2017 May;3(3):402–9.

7. Cummings J, Lee G, Ritter A, Zhong K. Alzheimer's disease drug development pipeline: 2018. Alzheimers Dement (N Y). 2018 May;4:195–214.

8. Herrup K. The case for rejecting the amyloid cascade hypothesis. Nat Neurosci. 2015 Jun;18(6):794–9.

9. Swerdlow RH. Is aging part of Alzheimer's disease, or is Alzheimer's disease part of aging? Neurobiol Aging. 2007 Oct;28(10):1465–80.

10. Prior M, Chiruta C, Currais A, Goldberg J, Ramsey J, Dargusch R, et al. Back to the future with phenotypic screening. ACS Chem Neurosci. 2014 Jul;5(7):503–13.

11. Bishop NA, Lu T, Yankner BA. Neural mechanisms of ageing and cognitive decline. Nature. 2010 Mar;464(7288):529–35.

12. Bredesen DE, Amos EC, Canick J, Ackerley M, Raji C, Fiala M, et al. Reversal of cognitive decline in Alzheimer's disease. Aging (Albany NY). 2016 Jun;8(6):1250–8.

13. Schubert D, Currais A, Goldberg J, Finley K, Petrascheck M, Maher P. Geroneuroprotectors: effective geroprotectors for the brain. Trends Pharmacol Sci. 2018 Dec;39(12):1004–7.

14. Hao H, Zheng X, Wang G. Insights into drug discovery from natural medicines using reverse pharmacokinetics. Trends Pharmacol Sci. 2014 Apr;35(4):168–77.

15. Swinney DC. Phenotypic vs. target-based drug discovery for first-in-class medicines. Clin Pharmacol Ther. 2013 Apr;93(4):299–301.

16. Yun UW, Yan Z, Amir R, Hong S, Jin YW, Lee EK, et al. Plant natural products: history, limitations and the potential of cambial meristematic cells. Biotechnol Genet Eng Rev. 2012;28:47–59.

17. Mushtaq S, Abbasi BH, Uzair B, Abbasi R. Natural products as reservoirs of novel therapeutic agents. EXCLI J. 2018 May;17:420–51.

18. Gunesch S, Schramm S, Decker M. Natural antioxidants in hybrids for the treatment of neurodegenerative diseases: a successful strategy? Future Med Chem. 2017 May;9(8):711–3.

19. Baell JB. Feeling nature's pains: natural products, natural product drugs and pan assay interference compounds (pains). J Nat Prod. 2016 Mar;79(3):616–28.

20. Tan S, Schubert D, Maher P. Oxytosis: A novel form of programmed cell death. Curr Top Med Chem. 2001 Dec;1(6):497–506.

21. Currais A, Maher P. Functional consequences of age-dependent changes in glutathione status in the brain. Antioxid Redox Signal. 2013 Sep;19(8):813–22.

22. Feng W, Rosca M, Fan Y, Hu Y, Feng P, Lee HG, et al. Gclc deficiency in mouse CNS causes mitochondrial damage and neurodegeneration. Hum Mol Genet. 2017 Apr;26(7):1376–90.

23. Fernandez-Fernandez S, Bobo-Jimenez V, Requejo-Aguilar R, Gonzalez-Fernandez S, Resch M, Carabias-Carrasco M, et al. Hippocampal neurons require a large pool of gluta-thione to sustain dendrite integrity and cognitive function. Redox Biol. 2018 Oct;19:52–61.

24. Sonnen JA, Breitner JC, Lovell MA, Markesbery WR, Quinn JF, Montine TJ. Free radical-mediated damage to brain in Alzheimer's disease and its transgenic mouse models. Free Radic Biol Med. 2008 Aug;45(3):219–30.

25. Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. Cell. 2012 May;149(5):1060–72.

26. Maher P, van Leyen K, Dey PN, Honrath B, Dolga A, Methner A. The role of Ca+2 in cell death caused by oxidative glutamate toxicity and ferroptosis. Cell Calcium. Forthcoming 2017.

27. Kang Y, Tiziani S, Park G, Kaul M, Paternostro G. Cellular protection using Flt3 and PI3Kα inhibitors demonstrates multiple mechanisms of oxidative glutamate toxicity. Nat Commun. 2014 Apr;5:3672.

28. Schubert D, Piasecki D. Oxidative glutamate toxicity can be a component of the excitotoxicity cascade. J Neurosci. 2001 Oct;21(19):7455–62.

29. Xie Y, Hou W, Song X, Yu Y, Huang J, Sun X, et al. Ferroptosis: process and function. Cell Death Differ. 2016 Mar;23(3):369–79.

30. Lewerenz J, Ates G, Methner A, Conrad M, Maher P. Oxytosis/ferroptosis-(Re-)emerging roles for oxidative stress-dependent non-apoptotic cell death in diseases on the central nervous system. Front Neurosci. 2018 Apr;12:214.

31. Saxena U. Bioenergetics failure in neurodegenerative diseases: back to the future. Expert Opin Ther Targets. 2012 Apr;16(4):351–4.

32. Winkler BS, Sauer MW, Starnes CA. Modulation of the Pasteur effect in retinal cells: implications for understanding compensatory metabolic mechanisms. Exp Eye Res. 2003 Jun;76(6):715–23.

33. Reshef A, Sperling O, Zoref-Shani E. Activation and inhibition of protein kinase C protect rat neuronal cultures against ischemia-reperfusion insult. Neurosci Lett. 1997 Nov;238(1-2):37–40.

34. Sperling O, Bromberg Y, Oelsner H, Zoref-Shani E. Reactive oxygen species play an important role in iodoacetate-induced neurotoxicity in primary rat neuronal cultures and in differentiated PC12 cells. Neurosci Lett. 2003 Nov;351(3):137–40.

35. Rego AC, Areias FM, Santos MS, Oliveira CR. Distinct glycolysis inhibitors determine retinal cell sensitivity to glutamate-mediated injury. Neurochem Res. 1999 Mar;24(3):351–8.

36. Sigalov E, Fridkin M, Brenneman DE, Gozes I. VIP-Related protection against lodoacetate toxicity in pheochromocytoma (PC12) cells: a model for ischemic/hypoxic injury. J Mol Neurosci. 2000 Dec;15(3):147–54.

37. Reiner PB, Laycock AG, Doll CJ. A pharmacological model of ischemia in the hippocampal slice. Neurosci Lett. 1990 Nov;119(2):175–8.

38. Maher P, Salgado KF, Zivin JA, Lapchak PA. A novel approach to screening for new neuroprotective compounds for the treatment of stroke. Brain Res. 2007 Oct;1173:117–25.

39. Chiruta C, Schubert D, Dargusch R, Maher P. Chemical

modification of the multitarget neuroprotective compound fisetin. J Med Chem. 2012 Jan;55(1):378–89.

40. McGeer EG, McGeer PL. Neuroinflammation in Alzheimer's disease and mild cognitive impairment: a field in its infancy. J Alzheimers Dis. 2010;19(1):355–61.

41. Lee YJ, Han SB, Nam SY, Oh KW, Hong JT. Inflammation and Alzheimer's disease. Arch Pharm Res. 2010 Oct;33(10):1539–56.

42. Butchart J, Holmes C. Systemic and central immunity in Alzheimer's disease: therapeutic implications. CNS Neurosci Ther. 2012 Jan;18(1):64–76.

43. Wyss-Coray T, Rogers J. Inflammation in Alzheimer disease-a brief review of the basic science and clinical literature. Cold Spring Harb Perspect Med. 2012 Jan;2(1):a006346.

44. Rock RB, Peterson PK. Microglia as a pharmacological target in infectious and inflammatory diseases of the brain. J Neuroimmune Pharmacol. 2006 Jun;1(2):117–26.

45. Garden GA, Möller T. Microglia biology in health and disease. J Neuroimmune Pharmacol. 2006 Jun;1(2):127–37.

46. Dringen R. Oxidative and antioxidative potential of brain microglial cells. Antioxid Redox Signal. 2005 Sep-Oct;7(9-10):1223–33.

47. Zheng LT, Ock J, Kwon BM, Suk K. Suppressive effects of flavonoid fisetin on lipopolysaccharide-induced microglial activation and neurotoxicity. Int Immunopharmacol. 2008 Mar;8(3):484–94.

48. Budni J, Bellettini-Santos T, Mina F, Garcez ML, Zugno AI. The involvement of BDNF, NGF and GDNF in aging and Alzheimer's disease. Aging Dis. 2015 Oct;6(5):331–41.

49. Mattson MP, Chan SL, Duan W. Modification of brain aging and neurodegenerative disorders by genes, diet, and behavior. Physiol Rev. 2002 Jul;82(3):637–72.

50. Abe K, Takayanagi M, Saito H. Effects of recombinant human basic fibroblast growth factor and its modified protein CS23 on survival of primary cultured neurons from various regions of fetal rat brain. Jpn J Pharmacol. 1990 Jun;53(2):221–7.

51. Keegan K, Halegoua S. Signal transduction pathways in neuronal differentiation. Curr Opin Neurobiol. 1993 Feb;3(1):14–9.

52. Sagara Y, Vanhnasy J, Maher P. Induction of PC12 cell differentiation by flavonoids is dependent upon extracellular signal-regulated kinase activation. J Neurochem. 2004 Sep;90(5):1144–55.

53. LaFerla FM, Green KN, Oddo S. Intracellular amyloid-beta in Alzheimer's disease. Nat Rev Neurosci. 2007 Jul;8(7):499–509.

54. Steele JW, Gandy S. Apomorphine and Alzheimer Aβ: roles for regulated α cleavage, autophagy, and antioxidation? Ann Neurol. 2011 Feb;69(2):221–5.

55. Wirths O, Multhaup G, Czech C, Blanchard V, Moussaoui S, Tremp G, et al. Intraneuronal Abeta accumulation precedes plaque formation in beta-amyloid precursor protein and presenilin-1 double-transgenic mice. Neurosci Lett. 2001 Jun;306(1-2):116–20.

56. Billings LM, Oddo S, Green KN, McGaugh JL, LaFerla FM. Intraneuronal Abeta causes the onset of early Alzheimer's disease-related cognitive deficits in transgenic mice.

Neuron. 2005 Mar;45(5):675–88.

57. Sopher BL, Fukuchi K, Kavanagh TJ, Furlong CE, Martin GM. Neurodegenerative mechanisms in Alzheimer disease. A role for oxidative damage in amyloid beta protein precursor-mediated cell death. Mol Chem Neuropathol. 1996 Oct-Dec;29(2-3):153–68.

58. Liu Y, Dargusch R, Maher P, Schubert D. A broadly neuroprotective derivative of curcumin. J Neurochem. 2008 May;105(4):1336–45.

59. Currais A, Quehenberger O, M Armando A, Daugherty D, Maher P, Schubert D. Amyloid proteotoxicity initiates an inflammatory response blocked by cannabinoids. NPJ Aging Mech Dis. 2016 Jun;2:16012.

60. Maezawa I, Hong HS, Wu HC, Battina SK, Rana S, Iwamoto T, et al. A novel tricyclic pyrone compound ameliorates cell death associated with intracellular amyloid-beta oligomeric complexes. J Neurochem. 2006 Jul;98(1):57–67.

61. Chen Q, Prior M, Dargusch R, Roberts A, Riek R, Eichmann C, et al. A novel neurotrophic drug for cognitive enhancement and Alzheimer's disease. PLoS One. 2011;6(12):e27865.

62. Currais A, Prior M, Dargusch R, Armando A, Ehren J, Schubert D, et al. Modulation of p25 and inflammatory pathways by fisetin maintains cognitive function in Alzheimer's disease transgenic mice. Aging Cell. 2014 Apr;13(2):379–90.

63. Currais A, Huang L, Goldberg J, Petrascheck M, Ates G, Pinto-Duarte A, et al. Elevating acetyl-CoA levels reduces aspects of brain aging. Elife. 2019 Nov;8:e47866.

64. Dias DA, Urban S, Roessner U. A historical overview of natural products in drug discovery. Metabolites. 2012 Apr;2(2):303–36.

65. Wink M. Modes of action of herbal medicines and plant secondary metabolites. Medicines (Basel). 2015 Sep;2(3):251–86.

66. Chan K, Hu XY, Razmovski-Naumovski V, Robinson N. Challenges and opportunities of integrating Chises medicine into mainstream medicine: A review of the current situation. Eur J Integr Med. 2015;7:67–75.

67. Chen YB, Tong XF, Ren J, Yu CQ, Cui YL. Current research trends in traditional chinese medicine formula: A bibliometric review from 2000 to 2016. Evid Based Complement Alternat Med. 2019 Mar;2019:3961395.

68. Ishige K, Schubert D, Sagara Y. Flavonoids protect neuronal cells from oxidative stress by three distinct mechanisms. Free Radic Biol Med. 2001 Feb;30(4):433–46.

69. Ehren JL, Maher P. Concurrent regulation of the transcription factors Nrf2 and ATF4 mediates the enhancement of glutathione levels by the flavonoid fisetin. Biochem Pharmacol. 2013 Jun;85(12):1816–26.

70. Maher P, Akaishi T, Abe K. Flavonoid fisetin promotes ERK-dependent long-term potentiation and enhances memory. Proc Natl Acad Sci U S A. 2006 Oct;103(44):16568–73.

71. Gelderblom M, Leypoldt F, Lewerenz J, Birkenmayer G, Orozco D, Ludewig P, et al. The flavonoid fisetin attenuates postischemic immune cell infiltration, activation and infarct size after transient cerebral middle artery occlusion in mice. J Cereb Blood Flow Metab. 2012 May;32(5):835–

43.

72. Maher P, Dargusch R, Bodai L, Gerard PE, Purcell JM, Marsh JL. ERK activation by the polyphenols fisetin and resveratrol provides neuroprotection in multiple models of Huntington's disease. Hum Mol Genet. 2011 Jan;20(2):261–70.

73. Currais A, Farrokhi C, Dargusch R, Armando A, Quehenberger O, Schubert D, et al. Fisetin reduces the impact of aging on behavior and physiology in the rapidly aging SAMP8 mouse. J Gerentol A Biol Sci. J Gerontol A Biol Sci Med Sci. 2018 Mar;73(3):299–307.

74. Maher P. The potential of flavonoids for the treatment of neurodegenerative diseases. Int J Mol Sci. 2019 Jun;20(12):3056.

75. Maher P. Protective effects of fisetin and other berry flavonoids in Parkinson's disease. Food Funct. 2017 Sep;8(9):3033–42.

76. Yousefzadeh MJ, Zhu Y, McGowan SJ, Angelini L, Fuhrmann-Stroissnigg H, Xu M, et al. Fisetin is a senotherapeutic that extends health and lifespan. EBioMedicine. 2018 Oct;36:18–28.

77. Currais A, Chiruta C, Goujon-Svrzic M, Costa G, Santos T, Batista MT, et al. Screening and identification of neuroprotective compounds relevant to Alzheimer's disease from medicinal plants of S. Tomé e Príncipe. J Ethnopharmacol. 2014 Aug;155(1):830–40.

78. Fischer W, Currais A, Liang Z, Pinto A, Maher P. Old age-associated phenotypic screening for Alzheimer's disease drug candidates identifies sterubin as a potent neuroprotective compound from Yerba santa. Redox Biol. 2019 Feb;21:101089.