Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.

CP2

Evidence Summary

Preclinical data suggest CP2 prevents neurodegeneration and delays reproductive senescence while increasing mitochondrial efficiency. Novel small molecules targeting complex I are under development.

Neuroprotective Benefit: Mitochondrial complex I activity in the brain appears to decline in AD. In mouse models of AD, CP2 treatment preserves cognitive function, improves mitochondrial function, decreases inflammation, and promotes autophagy.

Aging and related health concerns: Mutations in complex I may be associated with longevity in humans. CP2 has not been tested in humans. In mouse models, CP2 treatment delayed reproductive senescence and prevented excessive weight gain.

Safety: CP2 is a research tool compound and has not been studied in humans. In mouse models, long-term CP2 treatment did not result in any physical or histopathological abnormalities.

What is it?

Tricyclic pyrone compounds including CP2 were originally synthesized based on the structure of pyripyropene A, a potent inhibitor of acyl-CoA:cholesterol acyltransferase 2 (ACAT2). ACAT inhibitors significantly reduce free cholesterol levels in cells by increasing ABCA1 expression and promoting cholesterol efflux.

CP2 is a cell-permeable tricyclic pyrone that crosses the blood-brain barrier and accumulates in neuronal mitochondria (Zhang et al., 2015). CP2 mildly inhibits mitochondrial complex I (NADH dehydrogenase) of the electron transport chain by competing with flavin mononucleotide for binding to the redox center. Mitochondrial complex I is the first rate-limiting step of intracellular respiratory activity and oxidative phosphorylation; it is a major contributor to the generation of the proton gradient across the mitochondrial inner membrane, which promotes ATP production. Mitochondrial complex I is a large enzyme complex consisting of 45 subunits, 7 of which are encoded by mitochondrial DNA and the remainder are encoded by nuclear DNA (reviewed in Sharma et al., 2009). Complex I contriburtes to the generation of reactive oxygen species. Mild mitochondrial stress induced by inhibition of complex I induces an adaptive stress response, which includes activation of the AMPK, leading to increased mitochondrial dynamics and function, improved glucose uptake and energy homeostasis, reduced oxidative stress and inflammation, and improved autophagy and proteostasis (Trushina et al., 2023).

CP2 has been studied in numerous preclinical models of Alzheimer's disease (Maezawa et al., 2006; Hong et al., 2009; Zhang et al., 2015; Stajakovic et al., 2021; Stajakovic et al., 2021; Panes et al., 2023).

Neuroprotective Benefit: Mitochondrial complex I activity in the brain appears to decline in AD. In mouse models of AD, CP2 treatment preserves cognitive function, improves mitochondrial function, decreases inflammation, and promotes autophagy.

Types of evidence:

- No clinical trials or meta-analyses testing CP2 in humans
- 1 meta-analysis examining the relationship between complex I biomarkers (levels or activity) and neurological conditions including Alzheimer's disease
- 2 observational studies examining complex I PET imaging in Alzheimer's patients
- 1 GWAS study of cell lines to examine PK and safety of CP2
- 6 laboratory studies

- Several review articles

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function: None available.

Human research to suggest benefits to patients with dementia:

No studies have tested CP2 treatment in people with dementia.

In a cross-sectional study of 30 amyloid- and tau-positive mild Alzheimer's patients, significantly lower levels of mitochondrial complex I activity (measured by the PET SUVR of [^{18}F]BCPP-EF, which binds to complex I) were observed in the medial and lateral temporal lobe, including the hippocampus and parahippocampal gyrus, frontal cortex, precuneus, and cingulate gyrus, compared with cognitively unimpaired individuals (Terada et al., 2022). Higher levels of mitochondrial complex I activity (measured by [^{18}F]BCPP-EF SUVR) were correlated with brain glucose metabolism (measured by FDG-PET SUVR) across the cerebral cortex (especially in the temporal, frontal, parietal and occipital areas), hippocampus, parahippocampal gyrus, precuneus, and posterior cingulate gyrus in the Alzheimer's patients. Higher levels of mitochondrial complex I activity in the left parahippocampal gyrus, anterior cingulate gyrus, and right lateral temporal area correlated with higher cognitive scores (measured by MMSE). Higher levels of mitochondrial complex I activity in the left parahippocampal gyrus also correlated with higher logical memory score (measured by the Wechsler Memory Scale-Revised logical memory delayed recall score). Mitochondrial complex I levels were not associated with amyloid burden (measured by PiB-PET SUVR). Because this was a cross-sectional study, the study was not designed to show within-subject changes.

In a related study of 32 amyloid- and tau-positive mild Alzheimer's patients, mitochondrial complex I activity (measured by the [^{18}F]BCPP-EF SUVR) was significantly lower in the medial and lateral temporal, parietal, frontal cortex, precuneus, cingulate gyrus, thalamus, and basal ganglia (Terada et al., 2021). Tau deposition (measured by [^{11}C]PBB3 binding potential) was significantly higher in the temporo-parietal regions, including the parahippocampus and hippocampus, in mild Alzheimer's patients compared to age-matched control subjects. A significant inverse correlation was found between mitochondrial complex I activity and tau deposition in Braak stage I-II regions of interest, suggesting that mitochondrial complex I impairment coincides with tau deposition. Mitochondrial complex I activity was not correlated with tau deposition or amyloid deposition (measured by PiB-PET SUVR) in the Braak stage III-IV or V-VI regions of interest.

A meta-analysis of a total of 125 studies evaluating mitochondrial complex I or IV levels in neurological diseases reported that deficits in complex I and IV were observed in neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease (Holper et al., 2019). Complex I and IV were assessed in peripheral blood, muscle biopsies (for Parkinson's but not Alzheimer's studies), or postmortem brain samples at the level of enzyme activity or subunits (mRNA or protein levels). There were 12 studies evaluating complex I levels in Alzheimer's disease (and 33 studies evaluating complex IV levels). In Alzheimer's disease, complex I enzyme activity in the temporal cortex (temporal/entorhinal cortex) was significantly lower compared with controls ($p=0.0023$). Blood levels of complex I subunits (NDUFA1/4/7-9, NDUFB2/3/6, and NDUFS3/4/5) were also significantly lower in Alzheimer's patients compared to controls ($p<0.0001$ for all). In Parkinson's disease patients, complex I enzyme activity in peripheral muscle ($p=0.000084$) as well as in the substantia nigra ($p=0.006$) was significantly lower compared to controls.

Mechanisms of action for neuroprotection identified from laboratory and clinical research:

CP2 has not been tested in people with dementia. CP2 inhibited mitochondrial complex I in mitochondria isolated from postmortem human cortical tissue (Stajakovic et al., 2021).

In mice, CP2 is blood-brain-barrier penetrant. An acute CP2 treatment (25 mg/kg in 20% PEG 400 and 5% dextrose water in PEG, oral gavage) in a mouse model of Alzheimer's disease resulted in brain levels of CP2 at ~62 nM (Stajakovic et al., 2021). The maximum concentration of CP2 in the brain was reached 30 minutes after oral CP2 and averaged at 263.9 ± 63.6 nM.

In a cell culture system, CP2 (2 µM) reduces oligomeric Aβ by over 95% and modestly reduces cytoplasmic Aβ40 deposits (Maezawa et al., 2006). CP2 has a very high binding affinity to Aβ40 ($Kd=5.05$ nM) and a high affinity to Aβ42 ($Kd=269$ nM). In aqueous solution, 1.5 equivalents of CP2 bind to 1 equivalent of Aβ40, and at a 1:1: molar ratio, CP2 almost completely prevents Aβ40 and Aβ42 oligomer formation. Cell viability assays showed that when CP2 and 50 nM of Aβ42 are co-administered, CP2 completely blocks neuronal toxicity in a primary cortical culture.

A follow-up study by the same group showed that CP2 directly binds to Aβ42 oligomers (Hong et al., 2009), a toxic species of Aβ (Sengupta et al., 2016). CP2 inhibits Aβ42 aggregation, disaggregates Aβ42 oligomers and protofibrils, and blocks Aβ42 fibrillations (Hong et al., 2009). In a mouse model of familial

Alzheimer's (5xFAD mice), 2 weeks of CP2 treatment via intracerebroventricular infusion (100 µM, 370 ng/d) decreased non-fibrillar (by 40%) and fibrillar (by 50%) Aβ species (Hong et al., 2009).

CP2 has also been tested in mouse models of Alzheimer's disease (Zhang et al., 2015). First, CP2 treatment (25 mg/kg/d) was started *in utero* and continued for 56 weeks in 3 mouse models of AD (APP, PS1, APP/PS1). CP2-treated mouse models of AD did not show cognitive impairment at any age tested (up to 56 weeks), in contrast to untreated mice that showed deficits at 30 weeks of age. When CP2 treatment was started at 2.5 months (presymptomatic) and continued for 4 months, AD mice displayed superior working memory and exploratory behavior compared to untreated counterparts, along with a ~50% reduction in Aβ plaques, a ~15% reduction in soluble Aβ42, and a ~70% reduction in p-tau (Ser 396/404). Memory protection was detected after 2 months of treatment.

In wild-type cortical neurons, Zhang et al. showed that CP2 treatment lowers basal oxygen consumption rate and augments spare respiratory capacity, conferring mitochondria the ability to produce additional energy under conditions of increased work-load or stress. CP2 increases mitochondrial capacity and reduces proton leak, which together suggest that the electron transport chain in neurons is tightly coupled in the presence of CP2, with enhanced bioenergetics reserve and ability to withstand stress. Some counterintuitive effects of CP2 in wild type and FAD mouse neurons include increased NADH in a dose-dependent manner (but unaltered cellular NAD^+ concentration), reduced ATP levels, increased AMP, and increased AMP/ATP ratio. Neuroprotective actions of CP2 in mouse models of Alzheimer's disease include restoration of mitochondrial trafficking in neuronal axons (both anterograde and retrograde), increases in synaptic (synaptophysin) and neurotrophic (BDNF) markers, and a two-fold increase in pAMPKα, the activated form of the energy sensor, AMPKα.

In a mouse model of AD (APP/PS1 mice), acute CP2 treatment (25 mg/kg in 20% PEG 400 and 5% dextrose water in PEG, oral gavage) improved brain glucose uptake (measured by FDG-PET) to levels comparable to age- and sex-matched non-transgenic mice (Stajakovic et al., 2021). In the same mouse model, chronic CP2 treatment (25 mg/kg, oral gavage) started at 14 months of age (after onset of neuropathology and cognitive impairment) and continued until 23 months of age significantly improved spatial memory and learning (measured by the Morris water maze), restored attention and non-spatial declarative memory (measured by novel object recognition), reduced hyperactivity (measured by the open field test), and increased strength and motor coordination (measured by rotarod and hanging bar). In APP/PS1 mice, chronic CP2 treatment also improved synaptic activity (measured by LTP), restored dendritic spine density/morphology (increased thin and mature spines, spine density, synaptophysin, PSD95, and BDNF), and improved mitochondrial dynamics in the hippocampus. CP2 treatment also

reduced Aβ-related pathology, inflammation (decreased levels of TNF-α, Iba1, IL-12, and G-CSF), and oxidative stress (decreased MDA levels), and promoted lysosomal biogenesis and autophagy (increased expression of TFEB, LAMP-1, and LC3B) in the brain and periphery. CP2 treatment also activates nuclear factor erythroid 2-related factor 2 (Nrf2), a transcriptional master regulator of responses against oxidative stress (Trushina et al., 2023).

Also in the same mouse model of AD (APP/PS1 mice), CP2 treatment (25 mg/kg, oral gavage) started at 10 months of age (after onset of neuropathology) and continued for 10 months halted progressive degeneration of locus coeruleus neurons (measured by cortical TH+ axon density, TH+ neuron number and volume in locus coeruleus)(Stajakovic et al., 2021).

Using data from the Accelerating Medicines Partnership in Alzheimer's Disease (AMP-AD) Target Discovery and Preclinical Validation Project, CP2 treatment in APP/PS1 mice resulted in 567 differentially expressed genes, of which 128 were also differentially expressed in human Alzheimer's disease (Stajakovic et al., 2021). CP2 treatment in APP/PS1 mice reversed the expression of 71 genes that were upregulated in both mouse and human Alzheimer's disease. Based on functional enrichment analysis, the 71 genes were involved in the regulation of the immune processes, inflammation, response to reactive oxygen species, and TNF production. CP2 treatment also reversed the expression of 57 deregulated genes that are involved in axonogenesis, glutamatergic synaptic transmission, nervous system development, and synapse assembly. Thus, CP2 treatment in APP/PS1 mice counteracted the Alzheimer's disease-associated transcriptional changes.

In the 3xTg-AD mouse model of Alzheimer's disease, CP2 treatment (25 mg/kg/day, in 0.1% PEG dissolved in drinking water ad lib) from 3.5 to 18 months of age significantly improved spatial learning and memory (measured by Morris water maze), improved synaptic function (measured by LTP), increased levels of synaptic proteins (increased p-Ser845 of AMPAR GluA1 subunit, p-Tyr1472 of NMDAR GluN2B subunit, synaptophysin, and PSD95), reduced p-tau (Ser202/Thr205 and Thr231), improved peripheral and cerebral glucose metabolism (measured by glucose tolerance test and FDG-PET, respectively), and increased energy homeostasis (Stajakovic et al., 2021). Chronic CP2 treatment restored cerebral glucose uptake in 3xTg-AD mice to levels comparable to non-transgenic age- and sex-matched control mice. CP2 treatment did not significantly affect spatial learning and memory in non-transgenic mice. In 3xTg-AD mice, CP2 treatment did not significantly decrease Aβ levels with some increase in soluble Aβ fractions.

In a mouse model of AD (APP/PS1 mice), CP2 treatment (25 mg/kg/day in 0.1% polyethylene glycol dissolved in drinking water ad lib) started at 9 months of age (after onset of cognitive decline and neurodegeneration) significantly reduced dendritic mitochondria-on-a-string and endoplasmic reticulum (ER)/unfolded protein response stress in the hippocampus, and improved mitochondrial biogenesis and turnover (measured by increased Tfam, Tfeb, Parkin, LC3b, Mfn1, Mfn2)(Panes et al., 2023). The mitochondria-on-a-string phenotype is associated with extensive interactions with ER membranes, forming multiple mitochondria-ER contact sites (MERCS), which are known facilitate abnormal lipid and calcium homeostasis, accumulation of Aβ and p-tau, abnormal mitochondrial dynamics, and apoptosis. Chronic CP2 treatment in APP/PS1 mice significantly reduced MERCS in the hippocampus to levels below those observed in age-matched (24 month-old) non-transgenic mice.

In wild-type mice, a single oral dose of CP2 (25 mg/kg) resulted in a maximum brain concentration of CP2 (mean=263.9±63.6 nM) after 30 minutes (Gao et al., 2021). The exposure of brain cells to CP2 after oral administration was significantly below the IC50 values established in primary neurons. The highest concentration of CP2 (mean=11.47±1.36 µM) in plasma was achieved 1 hour after oral CP2.

CP2 has also been examined in *in vitro* models of Huntington's disease. In wild type cell culture systems, CP2 completely eliminated the formation of inclusions (protein aggregates) in neurons caused by the expression of the mutant huntingtin protein (mhtt) (Trushina et al., 2009). CP2 also reduced mhtt-induced aggregate formation in glial cells by 90% relative to untreated cells. In neurons from a Huntington's disease mouse model, CP2 restores caveolin-related endocytosis that is inhibited by mhtt expression, and prevents accumulation of neuronal cholesterol caused by mhtt. These CP2 effects on cholesterol are seen in Huntington's disease neurons but not in wild type neurons.

***APOE4 interactions**:* Unknown.

Aging and related health concerns: Mutations in complex I may be associated with longevity in humans. CP2 has not been tested in humans. In mouse models, CP2 treatment delayed reproductive senescence and prevented excessive weight gain.

Types of evidence:

- 1 meta-analysis examining the relationship between complex I biomarkers (levels or activity) and aging
- 1 observational study of mtDNA mutations and human longevity

- 0 clinical trials that are not in the meta-analyses/systematic reviews
- 0 observational studies
- 3 laboratory studies

No clinical trials have tested CP2 treatment in humans.

In an observational study examining the relationship between mitochondrial DNA and human longevity, mutations in complex I that resulted in partial loss of its activity were associated with a beneficial effect on longevity (Raule et al., 2014). However, simultaneous mutations in complex I and III or mutations in complex I and V were associated with detrimental effects on longevity.

CP2 treatment prolonged fecundity up to 14 months of age in both wild-type mice and mouse models of familial Alzheimer's disease (compared to ~8-10 months in normal mice without CP2 treatment) (Zhang et al., 2015). With aging, a mouse model of Alzheimer's disease treated with CP2 maintained their body weight similar to nontransgenic mice, while untreated mice got much heavier.

In neurons, CP2 provides greater resistance to H_2O_2-induced oxidative damage in a dose-response manner. Almost 100% of neurons survived in the presence of 5 uM of CP2, compared to 50% survival in the absence of CP2 (Zhang et al., 2015).

A study performed a longitudinal transcriptome analysis in a short-lived killifish, *N. furzeri*, and identified complex I as a hub for a negative correlation with lifespan (Baumgart et al., 2016). Partial pharmacological inhibition of complex I by rotenone at a very low concentration (0.1% of LC50) extended lifespan of *N. Furzeri* by 15%. However, lifespan was significantly shortened at a higher concentration of rotenone (1.0% of LC50). These studies suggest that the key is to only mildly inhibit complex I.

A meta-analysis of biomarker studies evaluating mitochondrial complex I or IV levels reported that both complex I and IV show robust age-related decline in humans (Holper et al., 2019). People over the age of 60 years old had lower complex I enzyme activity in the frontal cortex ($p<0.0001$) and muscle ($p<0.0001$) compared to people younger than 60 years old. There were 7 studies evaluating complex I in aging (and 14 studies evaluating complex IV).

Safety: CP2 is a research tool compound and has not been studied in humans. In mouse models, long-term CP2 treatment did not result in any physical or histopathological abnormalities.

Types of evidence:

- No clinical trials or meta-analyses testing CP2 in humans
- 1 GWAS study of cell lines to examine PK and safety of CP2
- 2 laboratory studies, 1 in mice and 1 on cell survival
- Several review articles

CP2 has not been tested in humans.

Mice treated with CP2 from *in utero* to 14 months of age (56 weeks) were well-groomed and displayed no physical abnormalities (Zhang et al., 2015). Histopathological examination also demonstrated a lack of developmental or other abnormalities.

In striatal neuronal cultures from Huntington's disease mice, cell survival assays testing CP2 concentrations from 0-40 µM showed that concentrations below 5 µM did not cause significant cell death but concentrations above 5 µM were toxic (Trushina et al., 2009).

In order to assess whether mild complex I inhibition could differentially affect people with specific genetic makeups, a genome-wide association study (GWAS) was conducted using a panel of ethnically diversified 196 human lymphoblastoid cell lines (Gao et al., 2021). This study found that CP2 administration is likely to be safe in the healthy population. There were 11 SNP loci and 64 mRNA expression probe sets that were possibly associated with CP2 susceptibility. Genes found to play a potential role in affecting CP2 cytotoxicity were related to drug metabolism; the expression of three genes was positively (CYP2A6, SPTBN4, and CHST11), and the expression of one gene (TXNRD1) was inversely associated with CP2 treatment sensitivity. The association of CP2 treatment with the expression of CYP2A6 varied based on the racial background. For example, cells from individuals of Han Chinese American background were more sensitive to CP2 treatment. Higher expression of genes involved in antioxidant response may increase CP2 tolerance. CP2 administration significantly altered only 3 genes related to mitochondrial function that could possibly be a concern toward the application of mitochondrial complex I inhibitors in humans (CISD3, CYB5RL, and cytochrome c oxidase subunit 2 [COX2]).

The maximum peripheral concentration of CP2 was close to the range of the lowest IC50 in lymphoblastoid cell lines, thus safety of chronic treatment with mild complex I inhibitors may need to be monitored in peripheral tissue where some toxicity may be anticipated in a small group of people with genetic susceptibility (Gao et al., 2021).

Drug interactions: Drug interactions with CP2 have not been studied to date.

Sources and dosing:

CP2 is a research tool compound not available commercially for human consumption. CP2 is synthesized based on methods previously described by Dr. Duy Hua at Kansas State University (Hua et al., 2003).

The only dosage information available is from mouse studies. AD model and non-transgenic mice were treated with 25 mg/kg/day in drinking water. The human equivalent dose that takes into account differential body surface area is 2.0 mg/kg/day for people weighing 60 kg. However, no safety or toxicity data are published in humans.

Research underway:

No clinical trials of CP2 are under way. With support from the ADDF, Harrington Discovery Institute and the NIH Blueprint program, the Trushina laboratory at the Mayo Clinic have developed novel small molecules that mildly inhibit complex I, with properties superior to CP2 (Trushina et al., 2023). As of the time of publication of this review article, the stage of drug development was focused on the hit-to-lead optimization and the identification of a preclinical drug candidate. The main focus of their program is to develop a safe and efficacious disease-modifying treatment for Alzheimer's disease. However, other diseases where this approach could be beneficial include schizophrenia, rheumatoid arthritis, polycystic kidney disease, and osteoarthritis.

Search terms:

Pubmed, Google:

- CP2
- CP2 + tricyclic pyrone
- Tricyclic pyrone
- Complex I

Clinicaltrials.gov

- CP2 (0)

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