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 but its efficacy and safety are unknown in humans.

Neuroprotective Benefit: Very promising for neuroprotection and potentially for Alzheimer's disease, but all evidence is based on preclinical work.

Aging and related health concerns: Delayed reproductive senescence and prevention of excessive weight gain with aging are seen in mouse models of Alzheimer's, but effects on other age-related health concerns have not been explored and there are no data in humans.

Safety: Safety and toxicity studies are unpublished and no clinical data are available.

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 (ETC) by competing with flavin mononucleotide for binding to the redox center.

Neuroprotective Benefit: Very promising for neuroprotection and potentially for Alzheimer's disease, but all evidence is based on preclinical work.

Types of evidence: (bullet points)

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

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. None available.

Mechanisms of action for neuroprotection identified from laboratory and clinical research. 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 an exceptionally 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), the most 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 familial AD (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).

The most extensive study on CP2 was carried out by Zhang and colleagues (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 FAD mice 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, FAD 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 pTau (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 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 ETC 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 FAD mice include restoration of mitochondrial trafficking in neurons, increases in synaptic (synaptophysin) and neurotrophic (BDNF) markers, and a two-fold increase in pAMPKα, the activated form of the energy sensor, AMPKα.

APOE4 interactions: Unknown.

Aging and related health concerns: Delayed reproductive senescence and prevention of excessive weight gain with aging are seen in mouse models of Alzheimer's, but effects on other age-related health concerns have not been explored and there are no data in humans.

Types of evidence:

- 0 meta-analyses or systematic reviews
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- 0 observational studies
- 3 laboratory studies

CP2 treatment prolonged fecundity up to 14 months of age in both wild-type and FAD mice (compared to ~8-10 months in normal mice) (Zhang et al., 2015). With aging, FAD mice treated with CP2 maintained their body weight similar to nontransgenic mice, while untreated FAD 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).

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.

Another 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.

Safety: Safety and toxicity studies are unpublished and no clinical data are available.

Types of evidence:

- 0 meta-analyses or systematic reviews
- 0 clinical trials that are not in the meta-analyses/systematic reviews
- 0 observational studies
- 2 laboratory studies, 1 in mice and 1 on cell survival

No data from humans are available. Mice treated with CP2 from *in utero* to 14 mo 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. Detailed safety and toxicity studies are not published.

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).

Sources and dosing: CP2 is 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), who owns the patent on CP2 (initial patent on tricyclic pyrones, #US5958970A, scheduled to expire on July 28, 2017).

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. However, no safety or toxicity data are published in rodents or in humans.

Research underway: No clinical trials are under way. CP2 is due to come off of patent in July 2017, making future commercialization challenging. Dr. Trushina has been funded by ADDF to identify and develop novel CP2-like compounds that are similarly effective in restoring mitochondrial function and protecting cognitive function. If successful, these compounds may generate new intellectual property.

Search terms:

Pubmed:

- CP2
- CP2 + tricyclic pyrone
- Tricyclic pyrone
- Trushina E
- Hua DH

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