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.

ENT1 Inhibitors

Evidence Summary

An ENT1 inhibitor has shown promise in preclinical models, though conflicting data with caffeine makes the results suspect.

Neuroprotective Benefit: There is some evidence from preclinical studies, but no clinical evidence, that ENT1 inhibitors may have some beneficial effects in neurodegenerative diseases.

Aging and related health concerns: Little evidence suggests that ENT1 inhibitors are useful for age-related diseases, and they may be detrimental when undergoing cancer therapy.

Safety: There is no evidence whether ENT1 inhibitors are safe in humans, though preclinical studies suggest they may have drug interactions with certain cancer or antiviral drugs.

What is it? ENT1 inhibitors may increase adenosine tone in tissues. Adenosine is a molecule that plays several roles in brain function. In neurons, it regulates cell survival and neurotransmitter release. In glia cells, it controls cell differentiation, astrogliosis, proliferation, and neurotransmitter uptake. Adenosine can be created intracellularly by conversion of AMP. In addition, ATP can be transported out of the cell where it is converted to AMP and then to adenosine. Extracellular adenosine is tightly linked to the energetic state of neurons, and its extracellular concentration reflects the depletion of intracellular ATP. High extracellular adenosine can occur in conditions of fatigue, hypoxia, and hypoglycemia. There are four adenosine receptors (A_1, A_{2A}, A_{2B}, and A_3) which belong to the G-protein-coupled receptor family. A_1R and $A_{2A}R$ are most prominent in the CNS with A_1R primarily regulating excitatory transmission at pre- and post-synaptic sites and $A_{2A}R$ fine-tuning synaptic plasticity (Cellai et al, 2018).

Adenosine tone in the brain is controlled by multiple proteins. Equilibrative nucleoside transporter 1 (ENTs) and concentrative nucleoside transporters (CNTs) mediate the transport of nucleosides, nucleobases, and therapeutic analogs across membranes. CNTs are sodium-dependent while ENTs are sodium independent. These transporters mediate the bi-directional flow of adenosine in cells depending on the adenosine concentration gradient across the plasma membrane. There are four primary ENTs (ENT1-4) with ENT1 and 2 being the most well characterized. ENT1 and 2 are located on the plasma membrane while ENT3 is located on intracellular membranes.

ENTs are ubiquitously expressed throughout the body. In the human CNS, ENT1 is enriched in the frontal and parietal lobes, thalamus, midbrain, and basal ganglia. The distribution of ENT1 correlates with the distribution of A_1R (Jennings et al, 2000).

Increasing adenosine tone in the brain may be beneficial in Alzheimer's disease by preferential activation of A_1R which could normalize hyperexcitability and excitotoxicity.

ENT1 inhibitors include nitrobenzyl mercaptopurine ribonucleoside, dipyridamole and dilazep, nitrobenzylthioinosine

Neuroprotective benefit: There is some evidence from preclinical studies, but no clinical evidence, that ENT1 inhibitors may have some beneficial effects in neurodegenerative diseases.

Types of evidence:

- One postmortem study in Huntington's disease and Alzheimer's disease
- Four preclinical studies in neurodegenerative diseases

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function?

None

Human research to suggest benefits to patients with dementia:

Expression of $A_{2A}R$ is increased in post-mortem tissue from Alzheimer's patients (Cellai et al, 2018).

Mechanisms of action for neuroprotection identified from laboratory and clinical research

J4 is an ENT1 inhibitor which prevents the uptake of adenosine into cells *in vitro* and increases extracellular concentration of adenosine *in vivo* in the brain. Chronic treatment of an Alzheimer's animal model with J4 (before the appearance of plaques) improved cognitive performance and reduced the size of amyloid plaques. It improved synaptic plasticity in hippocampal slices and normalized the levels of synaptic proteins in the hippocampus ($pGluR1^{Ser831}$, NR2B, NR2A) but not the cortex. Finally, J4 treatment normalized the upregulation of adenosine receptor A_{2A} in astrocytes, but it had no effect on levels of inflammatory cytokines (Lee et al, 2018).

These results are counterintuitive as caffeine, a non-selective adenosine receptor competitive antagonist, has shown beneficial effects for Alzheimer's disease. In addition, overactivation of $A_{2A}R$ in preclinical models can lead to memory deficits (Cellai et al, 2018).

Huntington's disease

In post-mortem tissue from Huntington's patients, ENT1 transcripts were elevated at the early stages of Huntington's disease but not at later stages (Guitart et al, 2016). In the CSF, patients with Huntington's disease had lower levels of adenosine and higher levels of adenosine monophosphate (AMP), and in patients, ATP levels were inversely correlated with the number of CAG repeats. Adenosine/ATP levels in the CSF were negatively correlated with disease duration. ENT1 and ENT2 are upregulated in the striatum of two mouse models of Huntington's disease, and upregulation correlated with disease duration. Genetic knockout of ENT1 in Huntington's mice had no effect on motor function or

extracellular adenosine levels, but it increased lifespan by 7.9%. Furthermore, chronic treatment with an ENT1 inhibitor (JM1907) improved motor function and increased lifespan by 5.7% (Kao et al, 2017).

Stroke
Middle cerebral artery occlusion (MCAO) in rats increased the expression of ENT1 in the hippocampus. Nitrobenzylthionosine (NBTI, an ENT1 inhibitor) administered after the ischemic event reduced infarct volume and improved neurological outcomes after MCAO. Treatment increased adenosine levels, pCREB expression, and reduced apoptotic cell death (Zhang et al, 2020).

APOE4 Interactions:
None reported

Aging and related health concerns: Little evidence suggests that ENT1 inhibitors are useful for age-related diseases, and they may be detrimental when undergoing cancer therapy.

Types of evidence:
- Reviews for cancer and antiviral drugs.
- 4 preclinical studies of ENT1 null mice or ENT1 inhibitors.

Cardiovascular
Researchers have proposed ENT1 inhibitors for cardioprotection after an ischemic event as ENT1 inhibitors may potentiate the effect of endogenous adenosine which can help cardiomyocytes replenish the supply of ATP and other essential adenine nucleotides. Though there is little evidence for their use (Yang et al, 2015).

In a model of myocardial infarction, ENT1 knockout mice had a smaller infarct size than wild type mice (Rose et al, 2010).

Diabetes
In vitro, treatment of primary mouse hepatocytes with the ENT1 inhibitor NBMPR induced hepatocyte glucose production. Glucose production with NBMPR was suppressed with metformin but not AICAR (an analogue of adenosine monophosphate). Tracing studies suggest this is due to the fact that ENT1 is a transporter for AICAR (Logie et al, 2018).

Bone density

ENT1 null mice show abnormal bone density with age. Some regions (such as the cervical spine and upper thoracic spine) have increased bone density while other regions (lower thoracic and lumbar spine and femur) have reduced bone density. Old ENT1 null mice also show reduced motor coordination with age (Hinton et al, 2014).

Cancer

ENT1 inhibitors may reduce the efficacy of nucleoside anticancer or antiviral drugs by preventing their uptake into tumor cells (Yang et al, 2015; Macanas-Pirard et al, 2017). In fact, one of the mechanisms for resistance to chemotherapeutics such as gemcitabine is a reduction of ENT1 in cancer cells (Ansari et al, 2012). This is not to say they increase the risk of cancer or viral diseases, but they should not be taken during cancer or antiviral therapy.

Safety: There is no evidence whether ENT1 inhibitors are safe in humans, though preclinical studies suggest they may have drug interactions with certain cancer or antiviral drugs.

Types of evidence:

- Multiple preclinical studies
- Reviews for cancer and antiviral drugs

Preclinical studies do not suggest that ENT1 inhibitors have significant safety issues, though they have never been tested in human studies (Lee et al, 2018).

Cancer

ENT1 inhibitors may reduce the efficacy of nucleoside anticancer or antiviral drugs by preventing their uptake into tumor cells (Yang et al, 2015; Macanas-Pirard et al, 2017). However, there is no evidence they increase the risk of cancer or viral infection.

Drug interactions:

ENT1 is a transporter for nucleosides and nucleoside analogs and will likely have drug interactions with certain cancer and antiviral drugs. In addition, other drugs that are taken up by ENT1 (such as AICAR) or that affect adenosine levels in the brain (such as caffeine) have interactions.

Sources and dosing:
Not available; currently in development

Research underway:
Currently in development

Search terms:

- Ent1 + alzheimer
- equibrilative nucleoside transporter 1 + inhibitor, lifespan, alzheimer, aging, cardiovascular, neuropathy, diabetes, hypotension
- nitrobenzylthioinosine + alzheimer, lifespan, aging, cardiovascular

Websites visited:

- Clinicaltrials.gov
- Pubmed