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.

Methotrexate

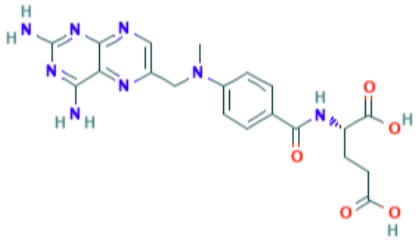
Evidence Summary

Evidence does not suggest that methotrexate would be beneficial for Alzheimer's disease or most patients with cardiovascular disease.

Neuroprotective Benefit: Methotrexate may be beneficial in patients with high systemic inflammation but does not target Alzheimer's-specific pathways.

Aging and related health concerns: Methotrexate is beneficial in patients with high systemic inflammation; however, an RCT does not suggest it would prevent cardiovascular events in most patients.

Safety: Methotrexate is associated with a number of well-known side effects including risk for increased liver enzymes in certain patients.

Availability: Available as a generic medication as an oral or injectable (intramuscular, intravenous, subcutaneous) drug.	**Dose:** 15-20mg once/week plus 1mg of folic acid daily	**Chemical formula:** $C_{20}H_{22}N_8O_5$; **Molecular Weight:** 454.44 g/mol **Source:** Pubchem 
Half life: 5-8 hours	**BBB:** Probably not	
Clinical trials: 484 ongoing.	**Observational studies:** 2 for dementia, 2 meta-analyses for cardiovascular disease	

What is it?

Methotrexate is an anti-folate drug that inhibits dihydrofolate reductase and is a first-line treatment for rheumatoid arthritis and other autoimmune diseases, such as psoriasis. It is administered once per week either orally, subcutaneously, or intramuscularly. It is administered weekly because methotrexate is transported into the cytoplasm through the reduced folate carrier where it is converted into polyglutamates. Polyglutamate formation ensures that methotrexate remains intracellular. Through a number of downstream effects, methotrexate inhibits the phosphorylation of NF-kB and the expression of TNFα and IL-6 (Ling et al, 2018).

As an analogue of folic acid, methotrexate inhibits the metabolism of folic acid. Supplementation of with 1mg folic acid daily is recommended with methotrexate treatment. Its anti-inflammatory mechanisms are thought to be due to an increase in adenosine levels (Tousoulis et al, 2016). There are large inter-individual differences in response to methotrexate. While these studies are in the early stages, response to methotrexate can be due to genetic, epigenetic, proteomic, and lifestyle factors (Ling et al, 2018).

Neuroprotective Benefit: Methotrexate may be beneficial in patients with high systemic inflammation but does not target Alzheimer's-specific pathways.

Types of evidence:

- 2 observational studies
- 1 in vitro study

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

A meta-analysis of 8 case-control studies and 2 longitudinal studies reported that individuals with rheumatoid arthritis were at a decreased risk of Alzheimer's disease (OR 0.6; 95%CI 0.46-0.77). However, a mendelian randomization study did not show a causal association between rheumatoid arthritis and Alzheimer's disease. The authors speculate this could be due to confounding factors such as rheumatoid arthritis patients taking anti-inflammatory drugs (Policicchio et al, 2017).

In a longitudinal study of individuals with rheumatoid arthritis, patients taking classical disease-modifying antirheumatic drugs (cDMARDs) were at a reduced risk of dementia compared to non-users (HR 0.60; 95%CI 0.42-0.85), an effect that was greatest for methotrexate users (HR 0.52; 95%CI 0.34-0.82) (Judge et al, 2017). On the other hand, in a study looking at Taiwan's National Health Insurance Research Database, patients with dementia were more likely to be taking methotrexate than those without dementia (OR 1.7; 95%CI 1.29-2.24) (Chou et al, 2017). One potentially confounding factor could be the case-control nature of Chou et al (2017). Patients with dementia may be given methotrexate due to the fact that an oral formulation of a drug may be easier to take than a biologic.

Both studies are confounded by the fact that they look at rheumatoid arthritis patients who are taking drugs versus those that are not. Rheumatoid arthritis may increase systemic inflammation, and it is not evident that patients without systemic inflammation would benefit from taking methotrexate.

Methotrexate is not predicted to cross the blood brain barrier.

Human research to suggest benefits to patients with dementia:

None

Mechanisms of action for neuroprotection identified from laboratory and clinical research:
Methotrexate inhibits folic acid synthesis and may increase homocysteine. An *in vitro* study suggested that incubation of hippocampal neurons in a folic acid-deficient medium or with methotrexate increased cell death and made neurons more vulnerable to AB-induced toxicity (Kruman et al, 2002).

APOE4 interactions:
None

Aging and related health concerns: Methotrexate is beneficial in patients with high systemic inflammation; however, an RCT does not suggest it would prevent cardiovascular events in most patients.

Types of evidence:

- 1 RCT in cardiovascular disease
- 2 meta-analyses of observational studies for cardiovascular disease in RA and psoriasis patients
- 2 observational studies of carotid-intima thickness
- 2 small RCTs of metabolic disease
- 2 preclinical studies of atherosclerosis

Cardiovascular disease
Paul Ridker, from Brigham and Women's Hospital at Harvard Medical School, discovered that healthy individuals with the highest quartile of baseline CRP had an increased risk for myocardial infarction (RR 2.9 p<0.001) and ischemic stroke (RR 1.9, p=0.02) over an 8-year follow-up. Participants were men in the Physicians' Health Study and part of an RCT that randomized patients to aspirin or placebo. Aspirin use was associated with a 55.7% reduced risk of myocardial infarction (p=0.02) in men in the highest quartile of CRP but only a 13.9% non-significant reduced risk in men in the lowest quartile of CRP levels (Ridker et al, 1997). In the Jupiter trial, a study testing the effects of rosuvastatin in healthy individuals with LDL levels less than 130mg/dL and hsCRP levels greater than 2.0mg/L, patients taking rosuvastatin for a mean duration of 1.9 years (max, 5.0) had a reduced rate of the primary endpoint (HR 0.56; 95%CI 0.46-0.69 – myocardial infarction (MI), stroke, arterial revascularization, hospitalization for unstable angina, or death from CVD). Rosuvastatin reduced LDL cholesterol by 50% and hsCRP by 37% (Ridker et al, 2008). These results suggested that high inflammation may also contribute to atherosclerosis.

Results from the first pure test of the inflammatory hypothesis of athersclerosis were announced in 2017 where individuals with previous myocardial infarction and hsCRP levels greater than 2mg/L were

treated with canakinumab (IL-1B inhibitor) over a mean follow-up of 3.7 years. Rates of the primary end point (nonfatal myocardial infarction, nonfatal stroke, or cardiovascular death) were reduced in the 150mg group (HR 0.85; 95%CI 0.74-0.98) and the 300mg group (HR 0.86; 95%CI 0.75-0.99) Fatal infection or sepsis increased from 0.18% in placebo to 0.34% in the 300mg group, but surprisingly fatal cancer decreased from 0.64% in placebo to 0.31% in the 300mg group (Ridker et al, 2017).

The Cardiovascular Inflammation Reduction Trial (CIRT) enrolled 4786 patients with a history of myocardial infarction or multivessel coronary disease and either type 2 diabetes or metabolic syndrome and treated them with 15-20mg methotrexate weekly over an average of 2.3 years. The trial did not meet its primary endpoint and did not reduce levels of hs-CRP, IL-6, or IL-1β. Baseline levels of hs-CRP were 1.52 mg/L (compared with >2mg/L in CANTOS). In the methotrexate group there was an increased incidence of infection, gastrointestinal disorders, non-basal-cell skin cancer, an increase in leukopenia, and an increase in liver enzymes (Ridker et al, 2018).

Epidemiology data also supports the beneficial effects of methotrexate (one caveat is that these studies are performed in patients with rheumatoid arthritis or psoriasis). One meta-analysis of 10 observational studies with 66,334 individuals reported methotrexate use was associated with a 21% lower risk of total CVD (95%CI 0.73-0.87) and a 18% lower risk of myocardial infarction (95%CI 0.71-0.96) compared to non-use (Micha et al, 2011). Another meta-analysis of 4 observational studies reported a 20% lower risk for CVD (95%CI 0.66-0.97) after adjusting for disease severity (De Vecchis et al, 2015).

In a small case-control study of patients with rheumatoid arthritis, Kim et al (2015) reported that patients whose drug regime included methotrexate had a smaller carotid intima-media thickness than those taking other drugs. However, another case-control study reported that individuals taking >20mg/week of methotrexate had a smaller carotid intima-thickness than those taking <20mg/week (Kisiel et al, 2015). An open label study reported that 6-week and 6-month treatment with methotrexate slightly increased HDL, LDL, and macrophage cholesterol efflux capacity (Ronda et al, 2015).

Preclinical studies

In a high-fat diet rabbit model where rabbits were given a sirolimus-eluting stent, weekly injections of methotrexate over 12 weeks resulted in an increase in arterial lumen size, decreased neointimal area and thickness, an increased fibrous cap, and decreased levels of inflammation(IL-6, MCP-1, TNFα, ICAM-1, VCAM-1 in serum and nuclear NF-kB in carotid tissue) (Zhang et al, 2016). In another study with cholesterol-fed rabbits, 4 weekly treatments of methotrexate resulted in a 75% reduction in lesion size,

a 2-fold reduction in intima-media thickness, a 50% reduction in macrophage migration into the intima, an 84% reduction in apoptotic cells, but no change in the proliferation of smooth muscle cells. In cell culture studies where human umbilical vein endothelial cells treated with TNFα, methotrexate downregulated pro-inflammatory genes and up-regulated anti-inflammatory genes (Bulgarelli et al, 2012).

Metabolic disease
In an open-label study of 24 patients with psoriasis, 12-week treatment with methotrexate (15mg/week) resulted in a 67% increase in endocan (which inhibits leukocyte adhesion), a 68% increase in the anti-inflammatory cytokine IL-10, a 50% decrease in CRP, but a 20% increase in triglycerides. There were no changes in HDL or glucose levels (Owczarczyk-Saczonek et al, 2018).

Cancer
Higher doses of methotrexate are used as a chemotherapeutic agent. It is not clear whether low-doses of methotrexate reduce the risk of cancer, although observational studies suggest that use of other rheumatoid arthritis reduce cancer risk more than methotrexate itself (Solomon et al, 2014).

Safety: Methotrexate is associated with a number of well-known side effects including risk for increased liver enzymes in certain patients.

Types of evidence:

- One review

Salliot and van der Heijde (2008) conducted a systematic review of 88 published studies of low-dose methotrexate monotherapy (avg 8.8mg/week) in rheumatoid arthritis patients up to 12 years in length. Long-term use of methotrexate did not increase risk for serious infections and reduced cardiovascular disease risk. Up to 13% of patients reported increased liver enzymes, and the data were mixed on evidence of liver fibrosis/cirrhosis. Other side effects are black stools, blood in urine and vomit, diarrhea, joint pain, reddening of skin, sores in mouse, stomach pain, or swelling of feet or lower legs (drugs.com). Methotrexate also inhibits folate synthesis, so folic acid should be supplemented.

Methotrexate should not be taken by people with alcoholism, cirrhosis, low blood cell counts, a weakened immune system, or pregnancy.

Drug interactions:
Methotrexate is associated with 202 major drug interactions including aspirin, other anti-inflammatory drugs, caffeine, everolimus, penicillin, other folate-depleting drugs, some anti-biotics, and others (drugs.com).

Sources and dosing:
15-20mg once per week; available as a generic.

Research underway:
There are currently 484 ongoing trials in various indications (clinicaltrials.gov).

Search terms:
methotrexate + alzheimer, dementia, longevity, aging, atherosclerosis, peripheral neuropathy, orthostatic, inflammation biomarker, cancer