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.

Methylene Blue (and TRx0237)

Evidence Summary

Low dose of reduced form of methylene blue shows some cognitive benefits. Phase III trials in Alzheimer's with TRx0237 show no evidence of slowing decline in memory and cognition, except possibly as a monotherapy.

Neuroprotective Benefit: Phase 2 trials of methylene blue showed some promise as a monotherapy. Phase 3 trials of TRx0237 in Alzheimer's patients show no evidence of efficacy as an add-on but possibly beneficial as a monotherapy.

Aging and related health concerns: No evidence that methylene blue is beneficial for human aging or age-related disease. Methylene blue extends maximum lifespan in female genetically heterogeneous (UM-HET3) mice.

Safety: Low-dose methylene blue has a good safety profile; however higher doses come with side effects such as anemia and serotonin toxicity.

What is it?

Methylene blue (methylthioninium chloride) is a tricyclic phenothiazine drug and dye acting as an inhibitor of nitric oxide synthase and guanylate cyclase. It is currently FDA-approved for the treatment of hereditary and acute methemoglobinemia, prevention of urinary tract infections in the elderly, and as a dye for intraoperative tissue visualization.

Most studies use methylene blue as methylthioninium chloride in its original state; however, the physiologically relevant state of the drug is the reduced form and human clinical trials in Alzheimer's have used both forms- leukomethylthioninium, LMTM or TRx0237, and oxidized methylene blue.

Neuroprotective Benefit: Phase 2 trials of methylene blue showed some promise as a monotherapy. Phase 3 trials of TRx0237 in Alzheimer's patients show no evidence of efficacy as an add-on but possibly beneficial as a monotherapy.

Types of evidence:
- 1 Phase II and 2 Phase III trials in mild-to-moderate Alzheimer's patients
- 1 trial on patients with claustrophobia and 1 trial on fMRI outcomes in healthy individuals using methylene blue
- Multiple preclinical studies in animal and in vitro models

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function?
No human studies have tested whether methylene blue prevents dementia or age-related cognitive decline. Preclinical studies suggest that it does cross the blood-brain barrier (1).

One randomized-controlledtrial (RCT) investigated the effects of methylene blue in 26 healthy subjects between the ages of 22-62 (2). Low dose (280 mg or ~4 mg/kg) methylene blue increased insular fMRI activity during a sustained attention and short-term memory tasks and significantly improved memory retrieval by 7% compared to placebo.

Another RCT examined the effect of low dose methylene blue (260 mg or 4mg/kg per day) on a fear extinction task in 42 subjects with claustrophobia (3). Results indicated that methylene blue administration improved fear extinction after one month follow up. It also improved contextual memory independent of its effects of fear extinction.

Human research to suggest benefits to patients with dementia or cognitive aging
A 24-week phase II dose-finding study in 321 patients tested the effect of methylene blue in patients with mild-to-moderate Alzheimer's disease not currently taking an acetylcholine (AChE) inhibitor or mematine (4). In patients with moderate Alzheimer's, a nominal dose of 138 mg/day (taken as 60mg tablets 3xday – 138mg based on estimated absorption) of methylene blue improved cognition (ADAS-cog scores dropped by 1 point from baseline, 5.42 points better than placebo). Likewise, there were improvements in ADCS-CGIC and MMSE scores compared to placebo and baseline. Mild patients, while having no cognitive benefits, showed an improvement in relative cerebral blood flow. Cognitive benefits were maintained during a 50-week extension on methylene blue. There were no benefits at the higher nominal dose of 228mg/day. There was no information in the publication of what kind of methylene blue capsule was used.

TauRx tested TRx0237, a derivative of methylene blue with superior pharmaceutical properties, with regards to solubility and PK, in a phase 3 study in 891 patients with mild-to-moderate Alzheimer's. Patients were treated with 75mg or 125mg of TRx0237 twice per day or a placebo (4mg of TRx0237 to control for urine discoloration) for 15 months. The drug failed to have any impact of cognition or function. However, a secondary analysis on patients taking the drug as a monotherapy showed significant treatment benefits in cognition (ADAS-Cog, ADCS-CGIC, MMSE) and function (ADCS-ADL) and decreased lateral ventricular volume. (5).

To further examine these treatment effects, TauRx modified the statistical analysis of an ongoing 18-month phase 3 trial to compare 100mg TRx0237 twice per day with a placebo (4mg TRx0237 twice per day) as well as the placebo (4mg TRx0237) as a monotherapy to an TRx0237 as an add-on therapy. Results from this study confirmed the secondary analyses of the previous study: patients taking TRx0237 had less decline in cognition, function, and glucose uptake than those taking TRx0237 as an add-on therapy. Additionally, those taking 100mg had more benefit than those taking 4mg (though the 4mg group had both monotherapy and add-on patients combined in the analysis). These studies suggest that TRx0237 may be beneficial as a monotherapy (6).

Being unable to compare these treatments head-on with the current approved drugs makes it unclear whether methylene blue is any better than the standard of care. The authors make some speculations about why the add-on therapies do not work, but they are not entirely sure.

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3

Mechanisms of action for neuroprotection identified from laboratory and clinical research
Methylene blue and TRx0237 treatment in animal models of Alzheimer's disease and tau suggest mixed results. Some show benefits when treatment is administered before the onset of cognitive deficits but not after. Some suggest they can reverse cognitive deficits. Mixed results suggest that methylene blue and TRx0237 may reduce tau levels, reduce amyloid beta, increase autophagy, increase proteasome activity, and improve cognition (7) (8) (9) (10; 11; 12).

Methylene blue reduces oxidative stress in Alzheimer's-like tau and β-amyloid aggregation *in vitro* models (13). In vitro, methylene blue reduces mitochondrial superoxide production and mitigates free radical formation (14). Methylene blue may influence downstream events of mitochondrial respiration including lipid β-oxidation, glycolysis, ATP synthesis, ECM production and Na+/K+ ATPase activity, thereby contributing to increased neuronal oxidative metabolic capacity (15). Methylene blue can readily cycle between oxidized (MB) and reduced (MBH_2) forms, thereby serving as an electron carrier within the electron transport chain in the mitochondrial matrix (16). Further, methylene blue treatment leads to increase in cellular O_2 consumption, ATP production and glucose uptake in primary astrocytes (17).

APOE4 interactions:
A phase 3 study reported no interactions with ApoE4 (6).

Aging and related health concerns: No evidence that methylene blue is beneficial for human aging or age-related disease. Methylene Blue extends maximum lifespan in female genetically heterogeneous (UM-HET3) mice.

Types of evidence:
- 1 lifespan study by the NIA's Intervention Testing Program
- Multiple lab studies in cell culture and 3D-organoid cultures

Details:
Although no clinical trials have tested the effects of methylene blue on aging and age-related disorders, several mechanisms have been proposed that provide some evidence for the beneficial effects on aging. The NIA's Intervention Testing Program (ITP) tested the effects of 27 mg/kg methylene blue in 4-month-old mice. Although there was no change in median lifespan in either sex, methylene blue significantly increased maximum lifespan by 6% in females compared to placebo (18).

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4

Methylene blue treatment is also shown to induce autophagy, both *in vivo* and *in vitro* (19)

Recent evidence in human skin fibroblasts from healthy donors and progeria patients (progeria is a disease of accelerated aging) showed that methylene blue attenuates reactive oxygen species in both groups. In human 3D skin organoid cultures, methylene blue improved skin viability, improved wound healing, increased skin hydration and dermis thickness (20).

Similar to multiple well-established interventions of aging, methylene blue activates AMPK, thereby inducing macroautophagy *in vitro* and in cortex and hippocampus of methylene blue treated mice, and it mitigates autoimmune encephalitis by activating AMPK/SIRT1 axis, inhibiting NF-κB and alleviating helper T-cell and T-regulatory responses (21; 22).

Safety: Low-dose methylene blue has a good safety profile; however higher doses come with side effects such as anemia and serotonin toxicity.

Types of Evidence
- 2 large RCTs in Alzheimer's disease
- Long-term use for methemoglobinemia

Methylene blue is on WHO's list of essential medicines with a high safety profile. Methylene blue follows a hormetic response, i.e. it is safe to consume in low doses but can be toxic at relatively higher doses of >5mg/kg. Since it is a monoamine oxidase inhibitor, intravenous administration can cause serious serotonin toxicity if combined with selective serotonin reuptake inhibitors, including duloxetine, venlafaxine, sibutramine, clomipramine or imipramine (23). Clinical trials report gastrointestinal side effects are most common. Methylene blue can cause a dose-dependent decrease in red blood cell count (4).

Individuals with Glucose-6-phosphate dehydrogenase (G6PD) deficiency should not be treated with methylene blue. Additionally, apart from common side effects like headache, sweating, nausea and limb pain, methylene blue can cause weakness and confusion, skin discoloration, blue-ing of urine, high serotonin levels causing hallucinations and fever (drugs.com – injectable; Medicine.net - oral).

Methylene blue can cause harm to the fetus when administered to a pregnant woman. Epidemiological studies suggest that methylene blue is a teratogen. The use of methylene blue in amniocentesis may be

associated with atresia of the ileum and jejunum, ileal occlusions and other adverse effects in the neonate (drugs.com).

Drug Interaction:
Methylene blue has many drug interactions. Especially important is that it should not be taken by individuals taking a serotonergic anti-depressant and many anti-psychotics:
https://www.drugs.com/drug-interactions/methylene-blue.html

Availability/Dosing:
The phase 2 study used a specialized capsule, but reported the best nominal dose was 138mg/day. The correct dosing for TRx0237 is still being worked out. As an antidepressant, 15 mg/day was identified as the potent dose of methylene blue (24). In the study by Rodriguez et al (2016) that showed an improvement in memory, 280mg (approximately 4mg/kg) of oral USP-grade methylene blue was used.

At high doses, methylene blue is shown to disrupt the mitochondrial electron transport chain and can be toxic. Non-pharmaceutical doses of methylene blue contain trace doses of arsenic, cadmium, aluminum, mercury and lead, which in high doses can be extremely toxic.

Research underway:
Currently, there is 1 ongoing clinical trial in the United States, investigating the effects of methylene blue in healthy aging, mild cognitive impairment and Alzheimer's disease with the primary outcomes being working and episodic memory task fMRI and response as a change from baseline with treatment for 2 or 12 weeks (NCT02380573). Additionally, TauRx Therapeutics recently completed a Phase III trial in an open label study using MB in AD patients, with the results discussed as before.

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