**BACKGROUND**

- Metadoxine is an ion-pair salt of pyridoxine (vitamin B6) and 2-pyrrolidone-5-carboxylate (PCA), also known as L-PGA, that has been used for more than 30 years to treat acute alcohol intoxication and chronic alcoholic liver disease.

- Pyridoxal phosphate−dependent enzymes are required for the biosynthesis of 4 key neurotransmitters: serotonin (5-hydroxytryptamine [5-HT]), epinephrine, norepinephrine (NE), and γ-aminobutyric acid (GABA).

- Although the exact mechanism of action of metadoxine is unknown, metadoxine extended-release (MDX101, MDX) demonstrated cognitive enhancing effects in a phase 2b study of 120 adults with attention-deficit/hyperactivity disorder (ADHD), and a phase 2b study of 36 adults with attention-deficit/hyperactivity disorder (ADHD). MDX is in clinical development for the treatment of ADHD (a 300-patient phase 3 study with adult ADHD is ongoing) and Fragile X syndrome (FXS).

- Improvements in cognitive function, working memory, and social interaction following treatment with metadoxine in a valid mouse model of FXS correlated with normalization of biochemical markers reflective of neuronal signaling pathways and oxidative stress.

- Key results are presented from a series of experiments designed to further characterize the mechanism of action of metadoxine.

**METHODS**

**Study Designs, Interventions, and Assessments**

- Behavioral assessments: In vivo behavioral testing (including contextual fear conditioning and various measures of social interaction) metadoxine was dissolved in saline (used as vehicle in all cases) and administered at an intraperitoneal dose of 150 mg/kg or 300 mg/kg/day for 7 days in 2-month-old WT mice to examine its potential for abuse.

- Brain biomarker assessments: The Ras-Mek-ERK and P38AK-mTOR signaling pathways are involved in mediating activity-dependent alterations in gene transcription underlying changes in synaptic function.

- Neurochemical assessments: The effect of metadoxine 150 mg/kg (ip) or (vehicle) once daily for 7 days in fmr1 KO (n = 6) and WT (n = 5) mice on brain biomarker levels was assessed. A brain-based microdialysis probe was inserted into the striatal region, including the ERK, Akt, and GABA-A receptor (GABA; a1, a5, and 5-HT).

- Electrophysiology assessments: The potential effects of metadoxine on striatal medium spiny neurons was evaluated by exposing horizontal brain slices (270 μm thickness) from 2-month-old WT mice to metadoxine (100, 200, and 300 μM) for 20 minutes and then measuring evoked postsynaptic responses to corticostriatal stimulation.

**RESULTS**

**Behavioral Effects of Metadoxine**

- Metadoxine 150 mg/kg/d IP and 150 and 300 mg/kg orally significantly reversed the freezing behavior deficit observed in fmr1 KO mice treated with vehicle for treatment effect, F = 15.34; df = 9; 90 (P < .0001)) (Figure 1A)

- Metadoxine also significantly reversed the social approach deficit observed in fmr1 KO mice (ANOVA for treatment effect, F = 19.3; df = 9; 9; P < .0001) (Figure 1B)

- There were no behavioral changes in WT mice treated with metadoxine.

**Neurochemical Effects of Metadoxine**

- In the microdialysis experiment, single-dose administration of metadoxine 150 and 300 mg/kg orally had no statistically significant effect on DA, 5-HT, or NE levels in the front cortex or on DA or 5-HT in the striatum of rats (data not shown).

- A single oral dose of metadoxine 70 mg/kg or 140 mg/kg had no acute neurobehavioral effects in vivo 1H-MRS metabolite levels (data not shown) or on striatal neurotransmitter levels (Figure 3).

**Electrophysiological Effects of Metadoxine**

- The electrophysiological effects of metadoxine suggest a dose-dependent reduction in the overall excitability of the corticostriatal network in striatal medium spiny neurons.

**CONCLUSIONS**

- In fmr1 KO mice, metadoxine improved performance on tasks involving learning, memory, and social interaction.

- These findings are consistent with results of clinical trials of MDX in human adults and children with FXS:
  - Efficacy of MDX was demonstrated for the entire patient sample
  - Preferential effects of MDX were demonstrated for patients with ADHD primarily inattentive subtype
  - Improvement in several neurobehavioral measures of attention and working memory was demonstrated

- Effects on exocytosis that was found in previous research, 5-HT, 5-hydroxytryptamine (5-HT), α1, α2, and 5-HT receptors are involved in mediating activity-dependent alterations in gene transcription underlying changes in synaptic function.

- The GABA assay demonstrated that the pharmacologic activity of metadoxine clearly differs from the activity of its individual components and from a 1:1 mixture of pyridoxine and PCA.

- Taken together, these findings reveal a novel mechanism of action of metadoxine characterized by monoamine independent GABA and glutamate modulation.

- The non-dopaminergic mechanism of action suggests that MDX may have a low potential for abuse.

**References**

6. On file. Alcobra Ltd. Tel Aviv, Israel.

**Disclosure Information**

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