A review of what is known about leucovorin and its relationship to autism

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In the beginning…

- 1999: the first description of the transport system for folic acid across the blood/brain barrier

Then Cerebral Folate Deficiency Disorder in 2002…

- Case report of a syndrome with "psychomotor retardation, spastic paraplegia, cerebellar ataxia and dyskinesia" +/- seizures was described in 5 children who were found to have normal serum folate levels, normal homocysteine but very low values of 5-methyltetrahydrofolate in CSF.
- Normal genetic coding of the folate receptor 1 gene
- Positive response to leucovorin with normalization of CSF 5-MTHF values and "amelioration" of symptoms.
- ???Related to autism

Then, 2005, antibodies against the primary folate receptor (FRα) were identified

- 28 children with **cerebral folate deficiency** (ages 2.4 to 19.3)
- 28 matched comparison children
- Blocking anti-bodies to FRα were found in 25 children with CFD
- Blocking anti-bodies to FRα were found in none of the comparison group
- Treatment with folinic acid ameliorated symptoms in the children under age 5 (not detailed in the article) with less improvement in the older individuals

Autistic symptoms in some children with CFD were identified in 2007

- Five of seven children with CFD were noted to have behavioral qualities consistent with autism
- Diagnosis of autism was made using the Autism Diagnostic Observation Scale and the Autism Diagnostic Interview-Revised

By 2008, there were published observations that low-CSF folate/ FRα antibodies were apparent in children with autism as well as those with CFD

- 25 children with low-functioning, early onset autism diagnosed by DSM-IV criteria
- 19 of 25 had blocking FRα antibodies while none of the comparison children had these antibodies
- The mean CSF 5MTHF level in the autism group was 27.3 while for the comparison group it was 82
- Folinic supplementation was given with **partial** improvement
- Currently, the positivity rate is quoted at 3.3% of children with developmental impairments other than autism
- The general population figure is 10 to 15%

And now, what you have been awaiting…

- Results of clinical trials, double-blind and otherwise
- As of 2025, there have been: 4 open label trials, 6 placebo-controlled trials (one was single blind) and 1 "self-controlled" treatment trial
- There were a total of 325 participants with autism received the described intervention
- Published studies were done in the US (7), China (2), France(1), India (1) and Iran(1)
- One study paired folinic acid with twice weekly injected methylcobalamine, a second study used B6 40 mg, folate and vitamin B12 500 micrograms and a 3rd study paired folinic acid and risperidone

  I have omitted these 3 from further discussion for clarity

Method of diagnosis of autism…

- DSM-IV (1 study/China #2)
- ADOS + CARS-2 (1 study/US #3),
- DSM-V (2 studies/ US #6, India, #9)
- ADOS (1 study/France #7)
- ADOS + ADI-R (2 studies/US #5,#11)
- ADOS +CARS + ADI-R (1 study/Singapore #10)

Raising the question of uniformity of autism diagnosis

Numbers represent the studies listed in the references

Dose of folinic acid…

- 400 micrograms twice per day x 3 months (#2)
- 0.5 mg/kg of folinic acid if FRα antibodies were negative, if +, begin with 0.5 to 1mg/kg but if no response in 6 months, increase to 2 mg/kg x total of 2 years. 10 mg dose 50 mg/day (#2)
- 5 mg of folinic acid twice per day x3 months. This averaged 0.48 mg/kg/day #7)
- 2 mg/kg/day of folinic acid x 3 months. Maximum dose 50 mg/day (3 studies#4,#9,#10)
- 25 mg/day; if no response 50 mg twice per day (1 study, #11)

Raising the concern of non-uniformity of dose

Numbers represent the studies listed in the reference

Why does folinic acid supplementation ameliorate CSF folate level

- Folinic acid can cross the blood-brain barrier by utilizing the **reduced folate carrier** (RFC), particularly when the primary transporter, the folate receptor alpha (FRα), is blocked.
- The RFC is less efficient in the rate of transport than FRα leading to the need for high dose folinic acid supplementation

Potential side effects of folinic acid…

- For children, side effects of folinic acid (leucovorin) are generally mild but can include behavioral changes, sleep issues, and gastrointestinal upset.
- The majority of studies reported very limited side effects. Sleep issues and irritability tended to improve with continued use. Gastrointestinal symptoms resolved with a reduction of dose.

Primary behavior/language outcome measure…

- ABC, CARS,ATEC and PEP-3 These are behavior rating scales (#2)
- CELF-preschool-2, CELF-4 and PLS-5 depending on the age of the participant. These are standardized language measures (#4)
- CARS at baseline and the end (#5, #9)
- ABC, SRS + PedsQL. These are all behavior rating scales (#6, #11)
- Overall ADOS score, subscores in communication and social interactions + ABC (#7)
- Expressive & Receptive One Word Picture Vocabulary Test, Vineland adaptive behavior scales, CGI(#10)
- Variability of instruments used to measure behavioral/language outcome
  - Numbers represent the studies listed in the references

Metabolic measures of outcome

- Vitamin B12
- Plasma Folic acid
- Glutathione level
- Blocking and binding FRAA titers

Changes in Language and Behavior

- **Study #2**: Significantly improved sociability, receptive language, affective expression and communication.
- **Study #4**:Improved verbal communication of 5 points (FRAA -) and 7 points (FRAA +). The measure used has a standard deviation of 15 points so this represents a modest change

The secondary outcomes with p value of .05 or greater included: daily living skills, stereotypic behaviors, inappropriate speech

Changes in language and behavior

- **Study # 5**: Up to 2 year trial of folinic acid with increased doses for children with +FRAA to 2mg/kg/day.
- Mean age 4.4 years
  - The outcome measure was the CARS (Childhood Autism Rating scale, a parent questionnaire). Results decreased for the treated children from a mean of 41.3 (severe) to moderate or mild scores (mean 34.4). The untreated group had no change in the CARs score
  - FRα antibodies were found in 75.6% of the participants (control and active treatment)

Changes in language and behavior…

- **Study #6**: Open label trial of use of 2 mg/kg/day for 12 weeks
  - Average age 16.3 years
  - No significant decrease in scores from the Aberrant Check List, or the Social Responsiveness Scale. Scores from the parent-reported Pediatric Quality of Life scale showed very little change.

Changes in learning and behavior…

- **Study # 7:** Randomized placebo-controlled trial using 5 mg of folinic acid twice per day for 3 months
- Age mean 2.3
- ADOS total score , reciprocal social interaction and communication scores were significantly ($p < 0.05$) improved. No improvement in SRS scores

Changes in language and behavior…

Study #9: Double-blind placebo-controlled trial using 2mg/kg/dayof folinic acid up to a maximum of 50 mg/day x24 weeks

- Changes in the Childhood Autism Rating Scale(completed by parents) showed a decrease of 3.6 pts vs 2.4 pts in the comparison group.
  - Mild to moderate symptoms 30-36.5 p=0.0001
  - Severe symptoms of ASD 37-60
  - The change is statistically significant but is it functionally significant

Related research…

- **Study #12:** Milk-free diet reduced FRα antibody titers in 12 children significantly from 2.08 pmol of FRα blocked to .35pmol in 3 to 13 months
- Re-exposure to milk again raised the titers
- This was similar for cow's, camel and goat's milk
- ? Use of a milk-free diet in conjunction with folinic acid for greater efficacy. Not yet the subject of a trial.

Related research…

- **Study#13**: Subgrouping children with autism based on the subtypes of FRα antibodies present
  - Binding antibodies: higher B12 levels;
  - Blocking antibodies: relatively better redox metabolism and inflammation markers; clinically better communication, less stereotyped behavior and fewer mannerisms (via appropriate standardized questionnaires)

What can be concluded?..

- That the presence of FRα antibodies is evident in 44 to 79% of children with autism evaluated for the quoted studies. Several comparison groups of children with developmental impairments of other etiologies had a rate of positivity of 3.3%
- Treatment with folinic acid has support for significantly improving the symptoms of cerebral folate deficiency disorder which also is associated with high levels of FRα antibodies
- There are a small number of studies of varying quality regarding the effect of use of folinic acid for children with **autism** that do show some improvement in symptoms or language impairment and some behavioral symptoms.

What can be concluded?…

- As shown, the studies have varied in:
  - Dose and duration of folinic acid use,
  - Differences in the evaluation(s)/criteria used to identify autism in the participants
  - Wide range of ages used in most studies with response favoring younger children
  - A variety of outcome measures used so that it is difficult to compare studies
    - The majority were standardized questionnaires completed by parents regarding behavioral symptoms with some standardized evaluations for language skills reported as well.
  - Although statistically significant changes were reported for some behavioral and language parameters, the changes may not be of great functional significance

Unresolved questions…

- Is there a relationship between milk-free diet and response to folinic acid?
- Are younger children more likely to benefit from folinic acid supplementation and at what age should it be implemented for optimal impact?
- What differences in pathophysiology separate cerebral folate deficiency disorder from autism ? Clearly, the symptoms overlap significantly but some are unique to CFD and it is more severe and progressive.
- How do these observations about the pathophysiology integrate with the well documented and numerous genetic abnormalities found in children with autism?

One physician's experience…

- I have been prescribing folinic acid for children with autism since 2020.
- 35-40 children who have varied widely in age.
- The dose of leucovorin was titrated up to 2 mg/kg/day for 6months. The initial dose was 2.5 mg twice per day for those under age 5 and 5 mg twice per day for those older than age 5.
- The presence of FRα antibodies were not obtained because of difficulty and cost of obtaining them, antibody prevalence of > 50% in most studies
- Attempted to obtain standardized language testing at baseline and 6 months
  - No significant difference demonstrated in the treated children, but most did not return for the 6 month evaluation.

One physician's experience

- Outcome : 8-10 children with modest improvement in speech skills and, perhaps irritability, per parent observation but not clearly outside the improvement seen with intensive ABA and other therapies

Obtaining FRα Antibody Titers

- Very few laboratories perform the testing
- A kit is sent on request
- It is an indirect measurement of the antibody titers
- Not covered by insurance
- Not drawn by laboratories, but they will do so for a fee
- Cost of the test is between $295 and $350 + blood draw fee

My current thoughts…

- There are clearly many questions, need for additional studies and limited number of participants in the studies of leucovorin for autism. However, there is support for a relationship between autism, CFD and low cerebral folate level as well as leucovorin's ability to correct the deficit in the CNS. There is reasonable data to suggest some benefit from improving central folate levels, but the amount of change and what qualities are most impacted is unclear.
- Low risk intervention with some potential for modest improvement.

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