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Folinic acid improves the score of Autism in the EFFET placebo-controlled randomized trial

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ABSTRACT

Autism spectrum disorders (ASD) are influenced by interacting maternal and environmental risk factors. High-dose folinic acid has shown improvement in verbal communication in ASD children. The EFFET randomized placebo-controlled trial (NCT02551380) aimed to evaluate the efficacy of folinic acid (FOLINORAL®) at a lower dose of 5 mg twice daily.

Nineteen children were included in the EFFET trial. The primary efficacy outcome was improvement of Autism Diagnostic Observation Schedule (ADOS) score. The secondary outcomes were the improvement in ADOS sub scores communication, social interactions, Social Responsiveness Score (SRS) and treatment safety.

The global ADOS score and social interaction and communication sub scores were significantly improved at week 12 compared to baseline in the folinic acid group ($P = 0.003$, $P = 0.004$ and $P = 0.022$, respectively), but not in the placebo group ($P = 0.574$, $P = 0.780$, $P = 0.269$, respectively). We observed a greater change of ADOS global score (−2.78 vs. −0.4 points) and (−1.78 vs. 0.20 points) in the folinic acid group, compared to the placebo group. No serious adverse events were observed.

This pilot study showed significant efficacy of folinic acid with an oral formulation that is readily available. It opens a perspective of therapeutic intervention with folinic acid but needs to be confirmed by a multi-center trial on a larger number of children.

1. Introduction

Autism spectrum disorders (ASD) are a group of developmental disorders characterized by deficits in reciprocal social interaction (inability to initiate and sustain relationships), verbal and non-verbal communication with restricted repetitive interests and activities. Multiple interacting factors are implicated in the pathology including maternal, environmental, nutritional and genetic risk factors. Folate is a cofactor for the one carbon metabolism (1-CM), which plays a key role in neural development during the embryonic and fetal period and the early years of life [1,2]. Women with neural tube defect pregnancy (NTD) or ASD children are not folate deficient and yet maternal intake of folic acid during pregnancy reduces the risk of both disorders [3,4].

Folate crosses placental and brain barriers through a complex transport that involves a high-affinity transporter, the FRα, and the Reduced Folate Carrier (RFC), whose affinity is about 1000 fold lower [5]. FRα autoantibodies (FRαAb) inhibit the FRα-dependent transport of folate across the placenta and blood-brain barriers [6,7]. The prevalence of FRαAb is higher in children with ASD, compared to age matched unrelated normal children [6,8,9]. Recently, one randomized double-blind placebo-controlled trial tested a non-marketed formulation of folinic acid at a high dose of 2 mg/kg for twelve weeks at a maximum dose of 50 mg per day [10]. This trial showed that folinic acid significantly improved verbal communication in ASD children recruited in the USA [10]. The long-term safety profile of high-dose folinic acid is not known [11]. The EFFET trial aimed to evaluate the efficacy of an already marketed oral formulation of folinic acid (FOLINORAL®) to treat ASD at

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a lower dose of 5 mg twice daily, in a country without food fortification.

2. Methods

EFFET trial (evaluation of efficiency of folinic acid in ADS children) was a randomized placebo-controlled trial (NCT02551380) conducted at the University Hospital of Nancy (France) in ASD children aged between three and ten years and treated for 12 weeks.

The study was approved by the Ethical Committee of the East of France (Comité de Protection des Personnes Est III) on 09/04/2015 (n ° 15.04.06) and by the ANSM French national agency (agence nationale de sécurité du médicament et des produits de santé, registration n ° 150314A-31) on 01/05/2015. It was registered in the European Clinical Trials Database (EudraCT) under the number: 2015-000955-25.

The study was designed as a double-blind placebo controlled trial, but was considered single-blinded because the placebo capsules, although very similar in appearance to FOLINORAL®, were manufactured separately. The primary efficacy endpoint was an improvement of the Autism Diagnostic Observation Schedule (ADOS) (module 1 and 2) score as described [12] calculated as the addition of the sub score communication (cut off 4 for autism and 2 for autism spectrum disorder; the sub score reciprocal social interaction (cut off for autism, 7; and for autism spectrum disorder, 4 4). The secondary endpoints were the improvement in the Social Responsiveness Scale (SRS) score [13] (cut off, 75 for autism; 59 for autism spectrum disorders) and treatment safety. Serum FRab were measured as previously described [14]. Vitamin B9 and B12 were measured by radioimmunoassay (RIA) (SimulTRAC-SNB). Homocysteine and other metabolic markers were measured by mass spectrometry (4000 QTrap).

Children with ASD were recruited from October 2016 to April 2018. Inclusion and exclusion criteria were similar to those of the trial performed in the USA [10]. Inclusion criteria included the diagnosis of ASD defined by ADOS score, documentation of language impairment, unchanged therapy 8 weeks prior to enrolment, intention to maintain ongoing therapies constant throughout the trial and no changes in therapeutic management in the 8 weeks preceding the study. Exclusion criteria included any treatment that may alter the metabolism of folate, including folate and vitamin B12 intake, vitamin or mineral supplementation exceeding recommendations, severe irritability, gastro esophageal reflux, any known renal or hepatic disease, child prematurity (<37 amenorrhea weeks) and lactose intolerance hypersensitivity. Syndromic ASD cases or genetic variants or chromosome aneuploidy were not included in the study. Genetic consultation was made for patients included in EFFET study with absence of Fragile X Syndrome and of abnormalities detected by Array comparative genomic hybridization.

We planned to study continuous response variables (ADOS and SRS scores) from age- and sex-matched pairs of study subjects. We tested the *a priori* hypothesis that the change in ADOS and SRS scores was greater for the folinic acid group as compared with the placebo group using a two-tailed *t*-test with a $P < 0.05$. Considering a difference in the response of matched pairs normally with a standard deviation of 2 and a real difference in the mean response of matched pairs of −2.5, we needed to study 7 pairs of subjects to be able to reject the null hypothesis that the response difference was zero with a probability (power) of 0.8. The Type I error probability associated with this test of the null hypothesis was 0.05.

We performed a Cohen's d test to compare effect sizes (mean differences) between week 0 and week 12 in folinic acid and placebo groups. We compared the week 12-baseline differences of ADOS and SRS scores between folinic acid and placebo groups using *t*-test. Other characteristics, with normal distribution at baseline, were compared by the *t*-test and those without normal distribution, by the Mann-Whitney *U* test. The statistical analyses were performed using STATA/SE (Stata Corp, Texas, USA) and MedCalc (v18.10.2, MedCalc Software, Belgium).

Anonymized data not provided in the article will be shared at the request of other investigators for purposes of replicating procedures and results.

3. Results

Nineteen children were included (Supplementary Table 1 and Table 1 and Fig. 1A), who received either 2 × 5 .mg folinoral or placebo. The mean dose of folinic acid was estimated to 0.48 .mg/kg/d (SD 0,10, range 0.29-0.63). No significant difference was found in the clinical and biological characteristics of children in the two groups recruited in the study (Table 1). The global ADOS score and reciprocal social interaction and communication sub scores were

Table 1
Trial outcomes according to assigned groups.

Characteristics:	Folinic acid	Placebo	P value‡
Evaluation at Baseline (week 0), mean (SD)			
ADOS score[a]	16.8 (4.4)	16.3 (3.2)	0.48
SRS score[a]	92.2 (13.6)	93.4 (16.0)	0.54
Evaluation at week 12, mean (SD)			
ADOS score	14.0 (5.0)	15.9 (3.7)	0.48
ADOS score week 12 −week 0	−2.8 (1.9)	−0.4 (2.2)	0.02
ADOS social interaction score week 12 −week 0	−1.8 (1.3)	0.2 (2.20)	0.019
ADOS communication score week 12 −week 0	−1.2 (1.3)	−0.4 (1.1)	0.02
SRS score	83.9 (14.6)	85.5 (16.3)	0.77
SRS score week 12 −week 0	−8.3 (13.4)	−7.9 (12.7)	0.10
Number of capsules missed during treatment	3.8 (4.9)	5.3 (2.8)	0.35
Adverse events, N (%)			
Behavior event	2 (22%)	2 (20%)	1
Viral infection	6 (67%)	7 (70%)	1
Sleep disorder	1 (11%)	0	1
Allergy	0	0	1
Digestive disorder	0	1 (10%)	1
Folate at week 12, mean (SD)			
Serum folate at week 12, nmol/L	124.1 (10.4)	31.4 (19,2)	0.04

*EFFET: Evaluation of the efficiency of folinic acid in children with autism spectrum disorders.
[a] Abbreviations: ADOS, Autism Diagnostic Observation Schedule; SRS, Social Responsiveness Scale.

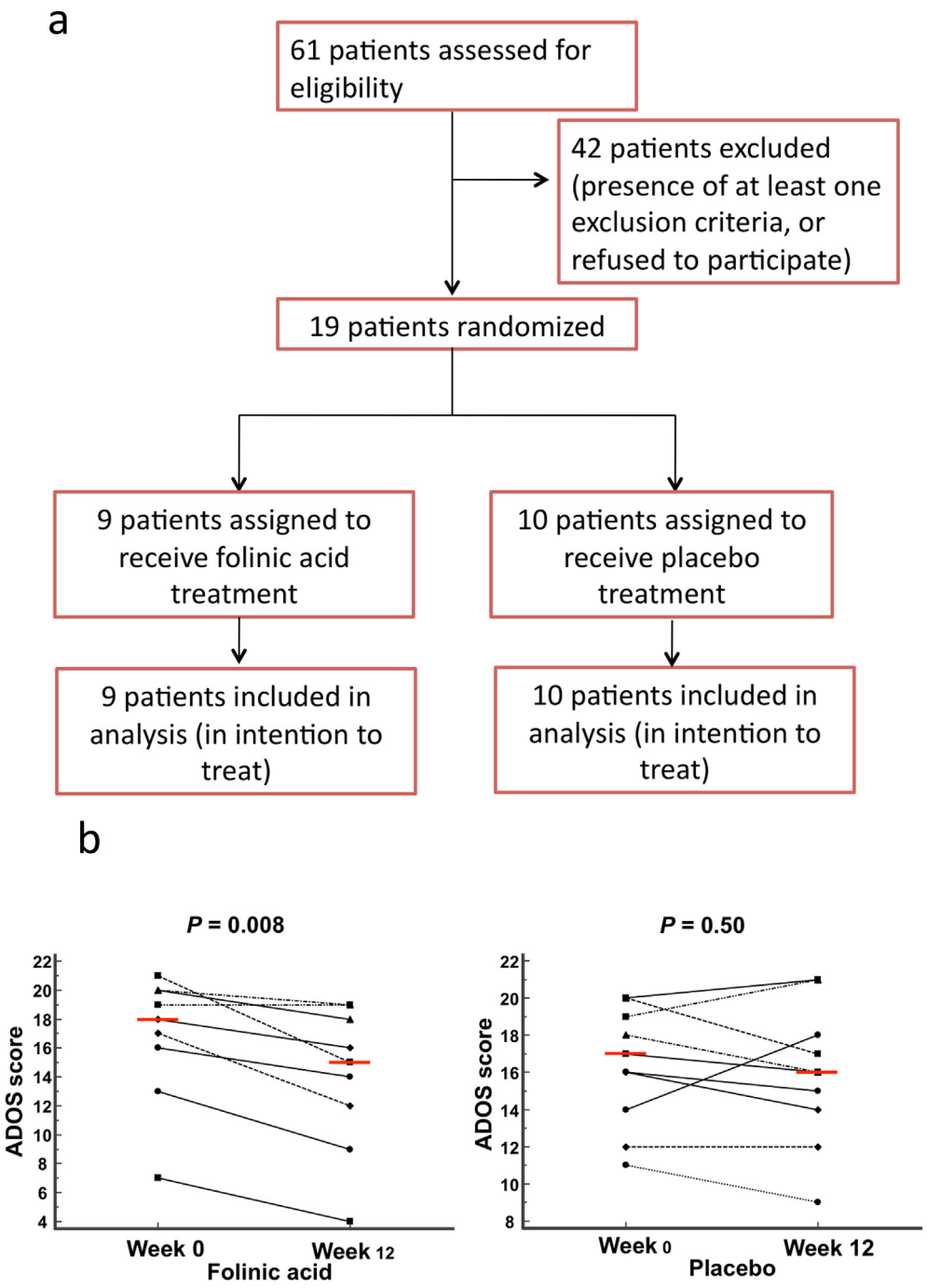

Fig. 1. A: Flow diagram of the EFFET Clinical Trial. The subjects excluded from the trial had similar sex and age distribution and no difference of Autism spectrum manifestations, compared to the trial subject. **B**: Change of Autism Diagnostic Observation Schedule (ADOS) global score in children with autism spectrum disorders, before and after 12 weeks of treatment with either folinic acid or placebo. The red lines indicate median values.

significantly improved at week 12 compared to baseline in the folinic acid group (P = 0.003, P = 0.004 and P = 0.022, respectively), but not in the placebo group (P = 0.574, P = 0.780, P = 0.269, respectively) (Table 1 and Fig. 1B). We observed a greater change of ADOS global score (−2.78 vs. −0.4 points, P = 0.020) and social interactions (−1.78 vs. 0.20 points, P = 0.019) in the folinic acid group, compared to the placebo group. The study reported no improvement in SRS score and no serious adverse events related to folinic acid intake (Table 1).

The frequency of FRαAb was similar in the folinic acid and placebo groups (Supplementary Table 1). There was no influence of the presence of either blocking or binding FRαAb with the change in ADOS scores after 12 weeks of treatment (P = 0.69 and 0.22, respectively).

4. Discussion

The EFFET trial on a small number of children with ASD demonstrated the efficacy of folinic acid for improving the global ADOS score and the social interaction and communication sub

scores at a dose of a readily available formulation of folinic acid widely used for other diseases. However, the conclusions of the study were limited by the sample population size.

In contrast to a previous trial conducted in the United States, we observed an improvement not only in the communication score but also in the social interaction score and the global ADOS score [10]. Social interaction is a sub-score of ADOS evaluation, whereas SRS is a questionnaire for parents. It is likely that the parents overstated the effects observed in their child and this may explain that no significant effect was reported on the SRS score. The frequency of FRα antibodies in our autistic group was similar to that reported in previous studies of autistic children (about 60%) [6,8], and significantly higher than in non-autistic population (about 10%). The consumption of bovine milk and milk products is associated with the production FRα antibodies [6,7,15]. However, we did not observe any association between FRα antibodies frequency and cow milk consumption in our patients. This lack of association can be due to the limited consumption of bovine milk and milk products by our cases and/or to the limited size of our study population.

Compared to the US trial, the EFFET trial observed a substantial effect of folinic acid despite the use of a 4-fold lower dose (Supplementary Table 2). The intake of folic acid is expected to be much higher in the US than in France, since France is a country without mandatory food fortification with folic acid [11]. The impact of prenatal folic acid on children's neurodevelopment and the risk of autism spectrum disorder (ASD) is a matter of debate. Two recent meta-analyses suggest that prenatal folic acid treatment is associated with a reduction in ASD risk [16,17]. The mandatory food fortification with folic acid could therefore introduce a difference in the type of ASD studied in the two trials. Other studies have raised concerns on the effect of high doses of folic acid on the neurodevelopment of children and the risk of ASD, recommending caution with excessive supplementation [18–20]. Another difference between the two trials was the mean age, with younger children recruited in our trial. Folinic acid treatment is expected to be more effective in the youngest children. Despite the lower dose used in the EFFET study (less than 0.5 mg/kg/day), it produced a 9-fold increase of serum folate concentration, compared to baseline concentration. This increase in folate concentration could improve the brain uptake via the FRα of methylfolate generated [8].

One other interesting point observed in our study is the absence of serious adverse events with folinic acid treatment in contrast with other treatments usually used in autism spectrum disorder [21].

5. Conclusion

In conclusion, our trial on a small number of children with ASD showed an association between folinic acid treatment and changes in ADOS scores at a safe dose of a widely used formulation. This pilot study opens a perspective of ASD therapeutic intervention that needs to be confirmed by a multi-center clinical trial on a larger number of children.

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Author contributions

E Renard, B Leheup and JL Guéant had full access to all of the data and take responsibility for the integrity and accuracy of the data analysis. Study concept and design: E Renard, B Leheup, EV Quadros and JL Guéant. Acquisition, analysis, or interpretation of data: All authors. Drafting of the manuscript: JL Guéant and E Renard. Critical revision of the manuscript for important intellectual content: All authors. Statistical analysis: RM Guéant-Rodriguez and A Oussalah. Obtained funding: JL Guéant and B Leheup. Study supervision: JL Guéant. E Renard, B Leheup and JL Guéant had full access to all of the data and take responsibility for the integrity and accuracy of the data analysis.

Declaration of competing interest

All authors with the exception of EVQ have no conflict of interest to declare. EV Quadros is an inventor in a patent for the detection of FRαAb issued to the Research Foundation of SUNY, NY, USA.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biochi.2020.04.019.

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