Rett syndrome: Neurologic and metabolic aspects
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Citation for published version (APA):

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Download date: 16 May 2019
Clinical and electroencephalographic effects of folinic acid treatment in Rett Syndrome patients

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Journal of Child Neurology 2011;26:718-723
Abstract

Rett syndrome is characterized by the development of stereotypic hand movements and seizures, which are often difficult to treat. Previous studies have shown conflicting results during add-on folinic acid. Here, the authors reevaluated the response to folinic acid in terms of epilepsy control and electroencephalography features. They performed a randomized, placebo-controlled, double-blinded crossover trial, with a follow up more than 2 year. Twelve girls with Rett syndrome participated, comparable in clinical stage and disease severity. The Rett syndrome patients were given either folinic acid or placebo, for 1 year each. Only 3 girls benefited to some extend: 2 had a reduction and/or decrease in seizures and all 3 of them showed some decreased epileptiform activity by electroencephalography during the addition of folinic acid. Despite this, antiepileptic drug were adjusted. Because the effect of added folinic acid was limited and did not prevent antiepileptic drug increase, the authors do not recommend adding on folinic acid in Rett syndrome girls with epilepsy.

Keywords: Rett syndrome, seizures, folinic acid, placebo-controlled study, electroencephalography, folate, epilepsy
Introduction

At present, Rett syndrome is considered to be a neurodevelopmental disorder in girls, as progressive neuropathological changes are generally lacking. Although motor performance slows with time, alertness, awareness and eye gaze-based communication remains throughout life.\(^1\) Clinical characteristics are abnormal deceleration of head growth, loss of speech and hand function and the development of stereotypic hand movements.\(^2\) Mutations in methyl-CpG-binding protein 2 gene (MECP2) may be found in 95% or more of Rett syndrome patients.\(^3,4\) At present Rett syndrome is a clinically, but not a genetically, defined disease with unknown pathogenic mechanism.\(^2\)

About 80% of Rett patients develop seizures over time. Epilepsy risk depends on the severity of the developmental problems, genetic mutation type, and age of the girl.\(^5,6\) In those younger than 2 years, seizures are not present. On the other hand, 33% of girls aged 3-5, 62% aged 5-10 and 86% aged 20-30 years, experiences seizures.\(^6\) In addition to seizures paroxysmal events like staring, hyperventilation and breath holding often occurs and might mimic seizures. To discriminate these events from non-epileptic events in Rett girls video-electroencephalographic (EEG) recordings are necessary.\(^7\) The disease severity of Rett girls has been classified in four stages\(^2\); EEG abnormalities correlates with the disease severity.\(^7\) Because EEG patterns can be classified in Rett girls, evaluation of EEG changes has an added value in reviewing seizure treatment. Seizures are often difficult to treat and a combination of 2 or more antiepileptic drugs is often needed.\(^5\)

Supplementation with folinic acid improved in contact, mobility and seizure control in some Rett syndrome girls but had no effect in others.\(^8-10\) Folinic acid might restore the low levels of 5-methyltetrahydrofolate (5MTHF) in the cerebral spinal fluid (CSF). Low levels of 5-methyltetrahydrofolate were shown to be present in some Rett syndrome girls\(^8\), but not in all.\(^9\) A recent study demonstrated no significant clinical effect of folinic acid supplementation.\(^11\) In these previous clinical trials, the use of folinic acid treatment was not placebo controlled or blinded \(^8,10,11\) and follow-up was often short.\(^11\) Detailed EEG evaluation, according to the Rett syndrome-EEG classification, to support any possible clinical effect, has not previously been described. Because of this controversy, we performed a two years prospective randomized double-blinded, placebo-controlled cross over study, to evaluate the effects of folinic acid treatment on seizure frequency and EEG changes in our Dutch Rett syndrome patients.
Patients and methods

We prospectively studied 12 Dutch Rett patients at the departments of Paediatric Neurology and Clinical Neurophysiology of the Academic Medical Center in Amsterdam, the Netherlands. Because some symptoms in Rett syndrome are progressive over time, like motor performances and epilepsy, and the ages of our patient are variable, we chose a randomized placebo-controlled, double blinded cross over study design. In that way, each patient is her own control. The study was approved by the medical ethics committee of the hospital. Written informed consent was obtained from all guardians of patients participating in the study.

Clinically diagnosed Rett girls of all ages, stage III (pseudo stationary period), stage III-IV (pseudo stationary period and non-ambulant < 10 years) and stage IV (late motor deterioration and non-ambulant)² participated in this study. In addition the modified symptom severity score (SSS) was used for more detailed severity classification. Individual clinical scores of 6 items, including onset of symptoms (maximum score 5), growth parameters (maximum 7), motor- (maximum 16), and communication function (maximum 8), Rett behaviour disturbances (maximum 9) and seizures (maximum 5) are summed up (score range 1-50). A higher score indicates increased severity.¹²

Exclusion criteria were the use of vitamin supplements or other research treatment of Rett syndrome, with an exception of anti epileptic drugs, and insufficient knowledge of the Dutch language by the caregivers.

Patients were randomly allocated to receive folinic acid during 1 year followed by placebo for 1 year, or the other way around. Intervening washout period was 2 months. So, total follow up time was at least 2 years and 2 months. The study consisted of 2 long treatment periods, to rule out temporary clinical or seizure frequency changes. All participating researchers and families were blinded for the study medication. The randomization was performed by our hospital pharmacy. Identical capsules of oral folinic acid (as calcium folinate) 2 mg/kg/d (maximum 50 mg/d), in combination with vitamin B₁₂ (cyanocobalamin) 0.5 mg/d or placebo were prepared and provided by the hospital pharmacy. Because folinic acid and vitamin B₁₂ are cofactors, vitamin B₁₂ is necessary for optimal action of folinic acid, so supplementation of both is required. Tolerability and side effects of folinic acid was assessed from parental reports and antiepileptic drugs were adjusted, as necessary.
Clinical and electroencephalographic effects of folinic acid treatment in Rett Syndrome patients

Seizure frequency and seizure types were recorded in the parental daily diaries. Video-EEG recording, 6 monthly, consisted of a 16 channel EEG (10-20 system). The EEG analysis consisted of background determination for (re)activity, epileptiform activity and (video) recording of seizures. Besides this, EEG of every patient was classified according to EEG staging, as previously described by Glaze et al. Briefly summarized, stage I consists of a normal or minimal slowing of the awake occipital dominant rhythm, stage 2 shows slowing of occipital-dominant and background rhythm and focal spike or sharp wave discharges, stage 3 dominant theta en delta activity and no occipital dominant rhythm, prominent rhythmic theta activity in central regions and loss of non-rapid eye movements sleep characteristics. Multifocal epileptiform discharges and generalized slow-spike-wave pattern appear more often during sleep and awake. At last, stage 4 demonstrates loss of occipital dominant rhythm, and marked slowing of the background activity (delta), multifocal epileptiform discharges in wake and almost continuous generalized slow-spike-wave activity during sleep. To quantify the amount of the epileptiform discharges in the EEG, a common Dutch classification was used according to the presence of epileptiform discharges: continuous, > 90% of EEG registration; frequent 50-90%; moderate 10-50%; sometimes 1-10%; and seldom < 1%. The EEG recordings were reviewed independently by the clinical neurophysiologist (JK) and a paediatric neurologist with clinical neurophysiologic certification (EH) under blinded conditions and consensus was reached.

Successful treatment was defined as either a 50% or greater reduction in seizure frequency over 6 months during treatment. An improvement in EEG background activity and/or EEG stage and a diminishment of epileptiform discharges in EEG were considered supportive for successful treatment.

Results

Patients Characteristics

In total, 12 Rett female girls participated in this study, of whom 10 girls (aged 2-11 years; mean 6.3 years), and 2 adult patients (table 1). Although the ages of the girls were variable, they showed similarity in clinical stage and SSS. Ten of them were classified as pseudo-stationary stage III (-IV) and 2 stage IV. Mean SSS was 22 (SD: 5; range 14-29). In all patients diagnosis were made clinically. MECP2 mutations were detected in 9 of these subjects (75%, results not shown). Patient 12 discontinued the study. Three patients were not treated according to the protocol
<table>
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<tr>
<th>No</th>
<th>Age yrs seizure onset</th>
<th>SSS</th>
<th>Clinical Stage</th>
<th>EEG-stage during change add on folinic therapy</th>
<th>Seizure or AED change during add on folinic therapy</th>
<th>Special EEG remarks during add on folinic therapy</th>
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<td>3</td>
<td>23</td>
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</tr>
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<td>24</td>
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<td>2/3 → 3</td>
<td>(cp, m, tc) seizures and SE ↓ ↑ VPA, LEV=, ad CLB placebo y</td>
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<tr>
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<td>11</td>
<td>4</td>
<td>29</td>
<td>IV</td>
<td>3</td>
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</tr>
<tr>
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<td>2</td>
<td>19</td>
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<td>2/3 → 3</td>
<td>(a, cp, t) seizures developed Start VPA + LTG</td>
</tr>
<tr>
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<td>6</td>
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<tr>
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<tr>
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<td>11</td>
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<td>26</td>
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<td>4</td>
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<td>8</td>
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<td>25</td>
<td>III-IV</td>
<td>4</td>
<td>(cp, t, m, SE) no data, LTG + LVT</td>
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</table>

SSS = symptom severity score
AED = anti epileptic drugs: CBZ = carbamazepine, CLB = clobazam, LTG = lamotrigine, LEV = levetiracetam, PB = phenobarbital, OXC = oxcarbamazepine, TPM = topiramate, VPA = valproic acid
a = absence, cp = complex partial, tc = tonic clonic, t = tonic, m = myoclonic, SE = status epilepticus; ↑ increase, = the same, ↓ decrease, ---- not present;
continuous (≥ 90% of EEG registration), frequent (50-90%), moderate (10-50%), sometimes (1-10%) and seldom (< 1%)
(patient 5 and 10 refused placebo, patient 1 only used folinic acid (blinded) for 2 years because of residence abroad). No side effects related to folinic acid treatment were reported.

**Seizures**

At study baseline 8 out of the 12 (67%) patients experienced seizures and 2 patients developed clinical seizures during follow up (patients 4 and 5). Thus, at the end of the study period 10 of the 12 patients (83%) were diagnosed with epilepsy (table 1). These 10 patients had various seizure types based on parental reporting, including absences (30%), complex partial seizures (70%), myoclonic seizures (30%), tonic-clonic seizures (70%) and tonic seizures (30%). Two patients experienced status epilepticus of tonic-clonic seizures and were hospitalised. One patient experienced seizure provoked by sound (no 2) and one at awakening (no 4), so called reflex seizures. In 8 out of 10 patients (80%), several types of seizures were identified.

In 3 patients (patient 1-3; 27%) antiepileptic drugs were discontinued (table 1). Patient 1 experienced monthly absence and tonic clonic seizures, that diminished by 50% during folinic acid treatment and because of this seizure reduction, 2 out of 3 antiepileptic drugs were discontinued. As a result, she was less sleepy and more active during the day. Patient 2 required, besides the adjustment of the doses of valproic acid, additional clobazam treatment during her placebo period, because she experienced almost daily severe tonic-clonic seizures and status epilepticus twice. During folinic acid treatment her seizure frequency decreased by 50%. She had 1 episode of status epilepticus. Instead of tonic-clonic and complex partial seizures she experienced more myoclonic seizures. Patient 3 had only 3 seizures during folinic acid treatment which was less than before and anti epileptic drugs were discontinued. Because no seizures reoccurred during the placebo therapy, the seizure control was probably not related to folinic acid use. Three of eleven patients (27%) developed seizures (patient 4-6) during folinic acid treatment. Patient 4 developed weekly complex partial and tonic clonic seizures for which anti epileptic drugs were started. Patient no 5, experienced monthly complex partial seizures at the age of 9 years, for which valproic acid was effective. She used folinic acid since the age of 6 year (not blinded), indicating that seizures may developed in spite of folinic acid treatment. During folinic acid treatment, lamotrigine had to be added to valproic acid in patient 6. In the remaining patients (patients 7-9)(27%) seizures and antiepileptic drugs were not changed, or seizures did not develop (patient 10 and 11; 18%). Patient 7 had 2 monthly seizures and patient 8 and 9 were seizure free.
Figure 1: Electroencephalography 10 seconds/page, Amplitude 50μV/cm. Patient no 2, age 5 years and clinical stage III-IV, EEG stage 3 during wakefulness. During Placebo. No obvious occipital dominant rhythm, slowing of background activity and central (temporal) epileptiform discharges and marked θ-activity.
Figure 2: Electroencephalography 10 seconds/page. Amplitude 50μV/cm. Patient no 2, age 6 years and clinical stage III-IV, EEG stage 3 during wakefulness. During folinic acid. No obvious occipital dominant rhythm, slowing of background activity and decrease of epileptiform discharges. The presence of β-activity is caused by the use of clobazam.
Electroencephalographic Characteristics

At least 5 EEGs were performed during the 2 years in 9 patients. In 2 patients (patient 8 and 10), fewer EEGs were performed due to logistical reasons. The EEGs of patients 1, 2 and 5 demonstrated the most obvious changes during folinic acid therapy. In patient 1 at baseline, the centro-temporal and/or parietal-occipital epileptiform discharges were almost continuously present (>90% of EEG time) and during repeated EEG recording frequently present (50-90% of EEG time). In patient 2, during placebo these epileptiform paroxysms were frequently present, but during folinic acid therapy they were less frequent (10-50% of EEG time). Besides this, both patients EEG showed improvement of background activity (figure 1 and 2). In patient 5, EEG recording started at the age of 3.5 years and showed frequent (50-90% of EEG time) centrottemporal epileptic discharges, without clinical events, and therefore AED was not started at that time. These epileptic discharges diminished during folinic acid therapy considerably and were only moderately present during 10 to 50% of EEG time. After 3 years of folinic therapy, at the age of 9 years, she experienced complex partial seizures and valproic acid was started, with which she became seizure free. Therefore, although a reduction of epileptiform discharges in the EEG was seen during folinic acid, it did not prevent the development of epilepsy in this girl.

In 2 girls (patient 4 and 6), the EEG deteriorated and changed to a higher (worse) EEG staging during folinic acid treatment, and in this period, both patients experienced severe seizures. No alterations of EEG staging or amount of epileptiform discharges were seen in the remaining 7 subjects (patients 3, 5, 7, 8, 9, 10, 11).

A correlation between the clinical and EEG stages was present in 9 patients (75%), but not in 3 patients without seizures, in which the EEG was better than expected from the clinical condition.

As expected, we frequently recorded (in 45% of our patients) non-epileptic events during simultaneous video-EEG recording, that clinically resembled seizures but without epileptic discharges. Non-epileptic events were upward deviation of the eyes, staring with head deviation and arrest, moaning and blue discolouring of the lips and hyperventilation and simultaneously staring.
Discussion

In this randomized, double-blind, placebo-controlled cross-over study with an extended long-term follow up, during folinic therapy, we demonstrated seizure reduction and/or decrease of epileptiform activity of the EEG in only 3 Rett syndrome patients. One other patient (patient 4) remained seizure-free on placebo treatment, which probably reflects the natural course of the epilepsy in this girl. In some patients antiepileptic drugs were adjusted at random. In the case of severe seizures, despite add-on folinic, antiepileptic drugs needed to be adjusted and/or added, for better seizure control. Therefore, the clinical effect of folinic acid seems to be very limited.

This is in accordance with previous studies. Improvement in the control of seizures and a decrease of epileptiform discharges have been demonstrated in a Spanish study of Rett girls 1 year after folinic acid supplementation. Folinic acid treatment was not blinded or placebo controlled in that study.\textsuperscript{10} These results could not be confirmed in a non-placebo-controlled prospective study, in which no EEGs results were reported.\textsuperscript{11} In the study of Temudo et al\textsuperscript{11}, only 44% of the Rett syndrome patients experienced seizures, whereas the prevalence of seizures in our study was 83%, comparable to previous reports.\textsuperscript{5,6} Two of our patients developed seizures while receiving folinic acid treatment and therefore it appears that folinic acid treatment does not prevent epilepsy. It is known that the epilepsy risk in Rett syndrome is age dependent, more common in those with more severe early developmental problems, and influenced by the mutation type.\textsuperscript{5,6}

At times, it may be difficult differentiating seizures from the behavioural patterns, often associated with Rett syndrome. Paroxysmal non-epileptic events such as episodes of breath-holding, hyperventilation, staring, jerky tremulousness, head turning, which mimic epilepsy, frequently occur in Rett patients.\textsuperscript{7} These non-epileptic events were seen in 45% of our patients and could only be differentiated from actual seizures, with the help of simultaneous video EEG recordings, as we performed.

The EEG is invariably abnormal after about 2 years of age and a relationship with the clinical stage of the Rett girl has been documented\textsuperscript{7} and was present in 75% of our girls (clinical stage III-IV). The corresponding typical EEG features, classified by Glaze et al, include slowing in background activity, rhythmic frontocentral theta activity, development of central spike or sharp wave discharges (EEG stage
3), and later more diffuse slowing and multifocal epileptiform discharges (EEG stage 4).\textsuperscript{7} Despite the presence of epileptiform EEG changes, clinical seizures may be absent in Rett syndrome girls.

To support our clinical data, long-time video EEG follow up was performed and demonstrated an improvement of the EEGs and reduction of epileptiform discharges in some Rett syndrome girls. For instance, patient 5, who used folinic acid and no antiepileptic drugs for several years, demonstrated reduction of epileptiform discharges. Detailed EEG follow-up during folinic acid treatment has not been previously reported, but is important to support the clinical efficacy of folinic acid therapy and essential to distinguish between epilepsy and paroxysmal non-epileptic events in our patients.\textsuperscript{7}

Currently, cerebral folate deficiency with a low level of 5-methyltetrahydrofolate and encephalopathy symptomatology has been described in the infantile-onset cerebral folate deficiency syndrome\textsuperscript{14} and mitochondrial encephalopathy’s.\textsuperscript{15} At the moment we do not know to what extent the presence of 5MTHF disturbances in Rett syndrome contribute to its pathophysiology, but it is likely to be a minor role. We did not see any adverse events related to folinic acid, such as nausea, behaviour and sleep disturbances. Most of our patients experienced severe seizures, despite multiple anti epileptic drugs. The impact of seizures on the Rett syndrome girls, their family and their quality of life is considerable.\textsuperscript{16}

Although there was some indication that some Rett syndrome girls seem to benefit from folinic acid, the clinical effect is very little and does not prevent the necessity for antiepileptic drugs adjustment in the case of severe seizures. Therefore, we do not recommend add on folinic acid in Rett syndrome girls with epilepsy.

Declaration of conflicting interests

Folinic acid was provided by the pharmaceutical company TEVA Pharmacemie, Haarlem The Netherlands. This company was not involved in study protocol design, follow up or study evaluation.
Literature Cited