Randomized open-label trial of dextromethorphan in Rett syndrome

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ABSTRACT

Objective: To determine safety and perform a preliminary assessment of dose-dependent efficacy of dextromethorphan in normalizing electrographic spikes, clinical seizures, and behavioral and cognitive functions in girls with Rett syndrome.

Methods: We used a prospective randomized, open-label trial in fast metabolizers of dextromethorphan to examine the effect of dextromethorphan on core clinical features of Rett syndrome. Interictal spike activity and clinical seizures were determined using EEG and parent reporting. Cognitive data were obtained using the Mullen Scales of Early Learning and Vineland Adaptive Behavior Scales, while behavioral data were obtained from parent-completed checklists, the Aberrant Behavior Checklist-Community Version, and the Screen for Social Interaction. Anthropometric data were obtained according to the National Health and Nutrition Examination Survey. The Rett Syndrome Severity Scale provided a clinical global impression of the effect of dextromethorphan on clinical severity.

Results: Dextromethorphan is safe for use in 3- to 15-year-old girls with Rett syndrome. Thirty-five girls were treated with 1 of 3 doses of dextromethorphan over a period of 6 months. Statistically significant dose-dependent improvements were seen in clinical seizures, receptive language, and behavioral hyperactivity. There was no significant improvement in global clinical severity as measured by the Rett Syndrome Severity Scale.

Conclusions: Dextromethorphan is a potent noncompetitive antagonist of the NMDA receptor channel that is safe for use in young girls with Rett syndrome. Preliminary evidence suggests that dextromethorphan may improve some core features of Rett syndrome.

Classification of evidence: This study provides Class IV evidence that dextromethorphan at various doses does not change EEG spike counts over 6 months, though precision was limited to exclude an important effect. *Neurology*® 2017;89:1684-1690

GLOSSARY

ABC = Aberrant Behavior Checklist-Community Version; **DM** = dextromethorphan polistirex; **MSEL** = Mullen Scales of Early Learning; **NKH** = nonketotic hyperglycinemia; **RL** = receptive language; **RSSS** = Rett Syndrome Severity Scale; **RTT** = Rett syndrome; **SSI** = Screen for Social Interaction; **VABS** = Vineland Adaptive Behavior Scales.

Rett syndrome (RTT), defined by mutations in the X-linked *MECP2* gene, manifests as post-natal microcephaly, intellectual disability, loss of purposeful hand use, seizures, and hypotonia that evolves to rigidity and scoliosis. Respiratory irregularities in the wake period are common. Repetitive stereotyped hand movements, seizures, and chaotic breathing are consistent with excessive excitatory activity in the brain, and levels of the excitatory neurotransmitter glutamate are elevated in the CSF of girls with RTT.^{1,2} We studied postmortem brain tissue from a 2-year-old girl with RTT and found marked elevation of excitatory NMDA receptors in the frontal cortex compared to controls.³ This finding was subsequently replicated in *mecp2*-null mice.⁴ The presence of spike activity on EEG in girls with RTT and the occurrence of clinical seizures in 50% are also consistent with hyperexcitability.⁵

Supplemental data
at *Neurology.org*

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Dextromethorphan polistirex (DM) (Delsym-Reckitt Benckiser LLC, Parsippany, NJ) and its major metabolite, dextrorphan, are potent noncompetitive antagonists of the NMDA receptor channel.⁶ DM is a commonly used antitussive agent that blocks the effects of excessive glycine, a co-agonist with glutamate at the NMDA receptor, which causes seizures in infants with nonketotic hyperglycinemia (NKH).^{7–9} It inhibits seizures induced by kindling and NMDA-induced convulsions in animal models and improves the EEGs of human epileptic patients.^{10,11} We administered DM in 3 different doses to test safety and efficacy. Spike activity was the primary endpoint. Secondary endpoints include clinical seizures, cognition, behavioral regulation, anthropometric measurements, and gait.

METHODS A prospective randomized, open-label trial was conducted to determine safety and perform preliminary assessment of the efficacy of DM for the treatment of the core features of RTT. These questions meet Class IV level of evidence. Girls between the ages of 2 and 14.5 years with RTT due to established mutations in the *MECP2* gene participated in this study.

Standard protocol approvals, registrations, and patient consents. The institutional review board of the Johns Hopkins University School of Medicine approved this study. The study was performed under an investigational new drug application with Food and Drug Administration FDR002408 and was reported in ClinicalTrials.gov NCT00593957. Informed consent was obtained from a parent of each patient.

Inclusion criteria. Inclusion criteria were presence of a mutation in *MECP2*; pre-enrollment EEG evidence of epileptiform discharges, with and without clinical seizures; and the ability to rapidly metabolize DM as determined by pharmacokinetics and pharmacodynamics studies.

Exclusion criteria. Exclusion criteria were patients without EEG evidence of interictal epileptiform activity; known history of diabetes or thyroid dysfunction; neurosurgical procedures for treatment of hydrocephalus and space-occupying lesions; medications that could potentially interact with DM, e.g., MAO inhibitors, selective serotonin reuptake inhibitors, and other drugs metabolized by the CYP450 isoform CYP2D6 (e.g., amiodarone, haloperidol, thioridazine); slow or intermediate metabolizers of DM; and individuals with a prior allergic response to DM.

The study was initially open to patients 5–15 years of age and was later extended to patients 2–5 years old after safety was established in 10 patients. Randomization to 1 of 3 doses was performed using the linear congruent algorithm of Park and Miller with Bays-Durham shuffling (Johns Hopkins Hospital investigational pharmacist; M.H.).¹² The patients were assigned to 0.25 mg/kg/d, 2.5 mg/kg/d, or 5.0 mg/kg/d dose of DM divided into 2 doses administered orally 12 hours apart. The 5.0 mg/kg/d dose was previously used in our NKH study and was well-tolerated. The 0.25 mg/kg/d was added as a pseudo placebo and the 2.5 mg/kg/d was selected as a middle dose.

Pharmacokinetics and pharmacodynamics analyses. Dextromethorphan is metabolized by CYP2D6, a cytochrome P450 enzyme to its active metabolite, dextrorphan. About 15% of Americans were found to be slow or intermediate metabolizers of dextromethorphan¹³ and the duration of action and effect of dextromethorphan in this group was increased up to threefold.¹⁴ Therefore, fast metabolizers were chosen to better control for duration of action and to minimize side effects. Dextromethorphan and dextrorphan measurements were obtained in plasma according to modification of a method from Ramachander et al.¹⁵ One milliliter of plasma containing 0.5 µg cyclizine hydrochloride (MP Biomedicals, Santa Ana, CA) used as an internal standard was incubated with β-glucuronidase type H-1 (1.0 mL of 3,000 units/mL solution in 0.1 M sodium citrate buffer, pH 5.0) at 37°C for 2 hours. This mixture was then adjusted to pH 10 with a saturated solution of sodium carbonate and extracted twice with 1.5 mL of ethyl acetate. The pooled extract was back-extracted into 2 mL of 0.1 N HCl, which was then adjusted to pH 10 and re-extracted with ethyl acetate. The extract was evaporated under nitrogen and derivatized with 75 µL N-methyl-N-(*t*-butyldimethylsilyl) trifluoroacetamide at 80°C for 60 minutes. Calibration curves using commercially available standards for dextromethorphan and dextrorphan (Sigma-Aldrich, St. Louis, MO) were extracted as above and quantification of both compounds was accomplished by single ion-monitoring gas chromatography/mass spectrometry. Patients were classified as fast metabolizers if the ratio of dextrorphan to dextromethorphan at 3 hours post dextromethorphan administration was greater than 15, intermediate metabolizers if the ratio was between 1 and 15, and slow metabolizers if the ratio was less than 1 (figure e-1 at Neurology.org).

DM safety. The safety of DM was determined based on patient questionnaire, complete blood count with differential, complete metabolic panel (electrolytes and liver function tests), DM levels, and EKG at 2 weeks and 1, 3, and 6 months (table e-1).

EEG and clinical seizures. Routine clinical overnight EEGs were performed before the initiation of DM and at the end of the 6-month study. We used either a Bio-Logic (Seyssinet-Pariset, France) Ceegraph system or a Compumedics (Victoria, Australia) Neuroscan system with Synamps amplifiers. A typical 10–20-electrode montage was used. The EEGs were reviewed using Persyst (Prescott, AZ) Insight II software (version 11). For each EEG, a trained research assistant marked epileptiform spikes and sharp waves for 2- to 5-minute periods in non-REM sleep. This was done to standardize brain states across analysis. The 2-minute period following sleep onset was excluded. A board-certified, fellowship-trained clinical neurophysiologist and pediatric neurologist (J.B.E.) verified all spike counts. Spike counts were averaged into per-minute rates. Seizure logs were maintained by the patient's caregiver documenting clinical seizure frequency beginning 1 month prior to the initial admission through the end of this 6-month study.

Neuropsychological assessment. A master's level psychometrist under the supervision of a doctorate level psychologist (R.V.) administered cognitive measures. These raters were blind to the patient's dose. Measurements were obtained at baseline, before drug administration, and at the end of study.

Cognitive status was assessed using the Mullen Scales of Early Learning (MSEL)¹⁶ and Vineland Adaptive Behavior Scales (VABS).¹⁷ The MSEL is designed to assess motor, cognitive, and language skills from infancy through preschool age. Since the girls enrolled in this study were outside of the typical age range for administration of this test, performance was reported

in age equivalents. Daily adaptive functioning was measured using parent-completed VABS.

Behavior characterization. Behavioral and temperament dysregulation were measured using the parent-completed Aberrant Behavior Checklist–Community Version (ABC)^{18–20} and the Screen for Social Interaction (SSI) to document current autistic-related behaviors.²¹ The ABC total score, comprising 5 subscales (irritability, agitation, and crying; lethargy/social withdrawal; stereotypic behavior; hyperactivity/noncompliance; and inappropriate speech) has good reliability and validity in individuals 5 years and older.¹⁹ The reliability and validity of the SSI total score and percentile score have been established for patients 30–60 months old.²¹

The Rett Syndrome Severity Scale (RSSS) is used to assess clinical severity and disease progression. Clinical severity has been correlated with reduced head circumference, reduced volume of specific brain regions on MRI, and decreased axonal *N*-acetylaspartate content on magnetic resonance spectroscopy.²²

Anthropometric measurement. Data were obtained as recommended by the National Health and Nutrition Examination Survey Anthropometry Procedure Manual. Height was measured

with hair ornament removed to the nearest 0.1 cm with a pediatric stadiometer (Ellard Instrumentation Limited, Monroe, WA) when recumbent length was required or with a standing stadiometer (Holtain Limited, Crymch, UK). Patients were weighed in light clothing to the nearest 0.1 kg with a digital scale (Scale-Tronix, White Plains, NY) either by themselves or minus parental weight. Head circumference was obtained at the occipitofrontal circumference level to the nearest 0.1 cm with the Seca (Quarryville, PA) 212 headband.

Gait. Ambulatory patients performed 5 walks across a GAITRite (Franklin, NJ) electronic walkway, at a comfortable speed to detect alterations in spatial-temporal measures of gait and provide quantitative or objective measures of gait.

Analysis. EEG spikes. Univariate analysis was performed using the Fisher exact test. Where the number of events was sufficient to justify multivariate analysis, logistic regression was used to control for the effect of potential confounders. Nonparametric Wilcoxon signed-rank test was used to test the difference in spike counts at each dose level between baseline and follow-up visits. Nonparametric Kruskal-Wallis test was also performed to determine if there was a statistically significant difference in spike counts across dose levels (0.25 mg/kg/d, 2.5 mg/kg/d, and 5 mg/kg/d) at each visit.

Figure 1 Randomization flow chart of Rett open-label dextromethorphan polistirex study

Clinical seizures. Nonparametric signed-rank test was used to compare clinical seizures between baseline and final visit at each dose. In addition, nonparametric Kruskal-Wallis test was used to determine statistically significant differences in clinical seizures across the 3 doses at each visit.

Behavioral characterization. Statistical analysis of the ABC was performed in a hierarchical manner such that the ABC subscales would be analyzed if the total ABC score analysis was statistically significant. The total ABC score and the SSI total score were first analyzed using baseline values compared with the 6-month follow-up values using the Wilcoxon signed-rank sum test. In order to determine the efficacy of each dose, the tests were performed on all patients together as well as stratified by dose (0.5 mg/kg/d vs 2.5 mg/kg/d vs 5 mg/kg/d).

Cognitive measures. Regression analysis was used to determine the association between scores at the follow-up visit and the dose level using a robust method for calculating the standard error. Sensitivity analysis was performed using the same regression model while adjusting for the baseline score while looking at the association between the follow-up score and dose level.

Rett Syndrome Severity Scale. The RSSS and its classification are shown in table e-2. Linear regression with robust standard error was used to determine the treatment effect (change in total RSSS) between the 2 visits. All 3 doses (0.25, 2.5, and 5 mg) were used as predictors in the linear regression.

RESULTS Demographic and clinical data. Forty-nine patients had a prerandomization EEG and pharmacokinetic studies of DM to determine eligibility. Of these, 11 were excluded because they were slow or intermediate metabolizers of DM. The remaining 38 patients were randomized to 1 of 3 dosage groups: 0.25 mg/kg/d, 2.5 mg/kg/d, or 5.0 mg/kg/d. Three of the 38 patients were not able to complete the study.

One patient on the 5-mg/kg/d dose was discontinued because of severe aspiration pneumonia after 3 weeks on DM. She had preexisting feeding difficulties and recurrent aspiration pneumonia. Another patient on the 5.0-mg/kg/d dose was discontinued after 2 months into the study because of drowsiness, intercurrent fever, diarrhea, urinary infection, and associated increase in liver function tests. The Data Safety Monitoring Board advised the discontinuation of DM despite the association of an acute infection with the increased liver function tests. A third patient was deferred and later dropped because of impending major surgery for scoliosis (figure 1). Thirty-five patients completed the study. There were no differences in age or mutation type for the patients between groups (table e-3).

Safety of DM in the treatment of RTT. All patients tolerated the drug and there were no hematologic, electrolyte, liver function, or EKG abnormalities related to DM use. In addition, parents denied type 1 diabetes, allergic skin rash, dizziness, drowsiness, urinary retention, ataxia, fatigue, dystonia, nystagmus, agitation, persistent nausea, vomiting, increased liver enzymes, and slurred speech.

Efficacy of DM for the treatment of RTT. EEG spikes. Data from 2 patients (1 on the 5 mg/kg/d and 1 on the 2.5 mg/kg/d dose) were excluded from the analysis due to lack of sleep. Another patient was excluded due to the presence of organized electrographic activity consistent with nonconvulsive status epilepticus,

Figure 2 Distribution of spike count

(A, B) Box plots distribution of spike counts at visits 1 and 2 by dose. (C) Difference in spike counts from visit 1 to visit 2 for each dose. IQR = interquartile range.

which was qualitatively different than the intraictal electrographic spikes and could not be analyzed quantitatively. There was no difference in the distribution of spike counts across doses for either visit 1 or 2 and no significant changes in spike count between the 2 visits for each dose (figure 2).

Clinical seizures. The effect of dextromethorphan on the clinical seizures was measured by comparing the number of seizures at the beginning and end of the study. Data from 2 patients in the 0.25 mg/kg/d dose and 2 patients from the 5.0 mg/kg/d dose were dropped from the analysis due to the absence of baseline or end of study data. Data from 3 patients, 1 in the 2.5 mg/kg/d dose and 2 in the 5.0 mg/kg/d dose, were also excluded from the analysis, as they experienced an increase in seizure frequency leading their attending physicians to change their anticonvulsant medication at different points in the study. Six of the 11 patients in the 0.25 mg/kg/d group were seizure-free for the duration of the study (figure 3A). There was a significant decrease in seizure frequency at the end of the study when compared to the beginning for the remaining 5 patients (figure 3B). Seven of the 11

patients in the 2.5 mg/kg/d group were seizure-free for the duration of the study while 3 of the remaining 4 had an increase in seizures at the end of the study and the last patient had a reduction in seizures (figure 3A). While there was some improvement on the 5.0 mg/kg/d dose, it was not statistically significant, as 4 out of 6 were seizure-free during the study and the remaining 2 had a reduction in the number of seizures (figure 3A). The 50% responder rate was 80% in the 0.25 mg/kg/d group, 25% in the 2.5 mg/kg/d group, and 100% in the 5.0 mg/kg/d group.

Cognitive function. Data from 25 patients in the MSEL and 29 patients in the VABS were analyzed due to incomplete responses on the questionnaire.

The regression analysis model estimates a significant increase in MSEL receptive language (RL) scores at follow-up by 6.1 points in the 5 mg/kg/d dose compared to the 0.25 mg/kg/d dose (tables 1 and 2). There was a 2.5-point increase in the 2.5 mg/kg/d dose compared to the 0.25 mg/kg/d dose but this was not-significant. Overall the dose has a significant association with MSEL RL score at follow-up after adjusting for baseline MSEL RL score ($p = 0.04$). There was no

Figure 3 Effect of dextromethorphan polistirex on clinical seizures

(A) Distribution of seizure counts by patients and treatment groups 1–3 over time. (B) Group difference in seizure counts from visit 1 to visit 2. IQR = interquartile range.

Table 1 Regression analysis of Mullen Scales of Early Learning (MSEL)

	Coefficient	SE	p Value	95% CI
Baseline MSEL	0.19	0.21	0.37	−0.22 to 0.59
Dose 2.5 vs 0.25 mg	2.46	1.97	0.21	−1.41 to 6.33
Dose 5.0 vs 0.25 mg	6.14	2.43	0.01	1.38 to 10.91
Constant	5.90	1.97	0.01	2.04 to 9.77

Abbreviation: CI = confidence interval.

Regression analysis output for MSEL: receptive language (RL). Multivariate regression analysis after adjusting for MSEL RL score at baseline to assess the association between MSEL RL score at follow-up visit and dose of medication.

significant effect of the dose on MSEL expressive language score after adjusting for baseline score ($p = 0.2$). There was no improvement in the VABS communication score when adjusted for baseline scores; however, there was a small but significant decline in score on the 2.5 mg/kg/d dose as compared to the 0.25 mg/kg/d dose.

Behavioral functioning. Using data obtained from the ABC, we calculated the change in the total ABC and subscale scores from visit 1 to visit 2. There was a significant improvement (decrease) in the total ABC score at the 2.5 mg/kg dose (median −6; interquartile range 14; $p = 0.03$) and the ABC hyperactivity subscale at the same dose (median −7; interquartile range 7; $p = 0.02$) but no improvement in the remaining subscales. While there was some improvement in social interaction as measured by the SSI scale, it was not statistically significant.

Clinical global impression. The linear regression model of the RSSS score between baseline visit and the second visit predicts that on average the change in total RSSS will be 0.4 points greater with the 2.5 mg dose compared to the 0.25 mg dose. Similarly, the change in total RSSS will be 1.1 points less in the 5 mg dose vs the 0.25 mg dose. Neither of these changes was statistically significant (table e-4).

Height, weight, and head circumference measurements were collected for 35 patients at their baseline and 6-month follow-up visits. The average growth of the patients during this period was 3.24 cm in length, 1.26 kg in weight, and 0.41 cm in head

circumference. Surprisingly, height and weight gains were consistent with that seen in healthy girls age 3–12 years.²³ Their head growth seemed to follow the growth curve; however, it is important to note that 29 of the 35 girls had baseline head circumferences below the 3rd percentile per the Rollins reference chart.²⁴ Head growth during the 6-month trial did not improve their percentiles. Nonparametric Kruskal-Wallis test analysis found no significant difference across doses for any growth measure.

Gait analysis was performed on all 9 ambulatory patients; however, due to the small number of patients in each group, statistical analysis was not performed.

DISCUSSION This prospective, randomized, open-label trial of dextromethorphan in girls with RTT is based on strong evidence that abnormal synaptic function is associated with excessive levels of glutamate in the brain and CSF and high levels of NMDA glutamate receptors in the cerebral cortex in postmortem RTT brain. Our data indicate that DM is safe and well-tolerated at all 3 doses when prescribed to girls with *MECP2* mutation who are fast metabolizers of DM. Although our primary endpoint of reduced spike activity was not achieved, dose-dependent improvement in some features of RTT, specifically RL, hyperactivity, and frequency of clinical seizures, was noted.

This study is limited by the lack of a placebo arm. Although the performance measurements used in this study are standard of care, they are not specifically designed to evaluate cognitive function in nonverbal individuals with poor hand use, making this a limitation. Nevertheless, our data are encouraging and warrant further study of DM in this population.

Randomized clinical trials of DM in girls with RTT are rare. Our results demonstrate that DM is not only safe in young children but may be of therapeutic value in the treatment of some core features of RTT. Thus it seems appropriate to conduct additional trials initiating DM soon after diagnosis in younger patients with RTT.

AUTHOR CONTRIBUTIONS

Constance Smith-Hicks: analyzed and interpreted the data, drafted and revised the manuscript. Siddharth Gupta: analyzed the data and revised the manuscript. Manisha Hong: generated the randomization, dispensed the study drug, and revised the manuscript. Lisa Kratz and Richard Kelley: performed dextromethorphan pharmacokinetic studies and revised the manuscript. Elaine Tierney: performed the behavioral test, analyzed the data, and revised the manuscript. Abanti Sanyal: performed statistical analysis and revised the manuscript. Gayane Yenokyan: provided guidance on statistical analysis and revised the manuscript. Josh Ewen: performed and analyzed the EEG and revised the manuscript. Nga Brereton: performed the anthropometric measurements and revised the manuscript. Michael V. Johnston: conceptualized the study, interpreted the data, and revised the manuscript. Sakubai

Table 2 Regression analysis of Vineland Adaptive Behavioral Scales (VABS)

	Coefficient	SE	p Value	95% CI
Baseline VABS: daily living skills	0.96	0.12	0.00	0.71 to 1.20
Dose 2.5 vs 0.25 mg	−1.61	0.75	0.04	−3.16 to −0.06
Dose 5 vs 0.25 mg	−1.36	0.99	0.18	−3.41 to 0.69
Constant	1.75	1.56	0.27	−1.47 to 4.97

Abbreviation: CI = confidence interval.

Regression analysis output for VABS: daily living skills.

Naidu: conceptualized and designed the study, interpreted the data, and revised the manuscript for intellectual content.

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DISCLOSURE

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