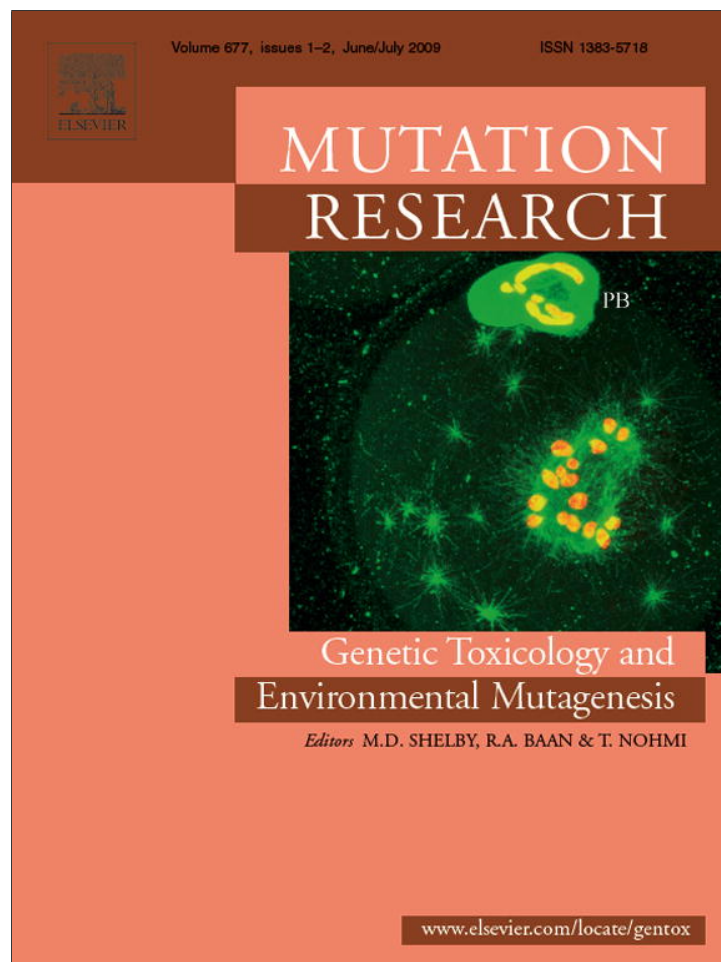


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Cytogenetic assessment of methylphenidate treatment in pediatric patients treated for attention deficit hyperactivity disorder

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ABSTRACT

Methylphenidate (MPH, Ritalin[®]), has been prescribed to treat attention deficit/hyperactivity disorder (ADHD) since its approval by the FDA over 50 years ago. Diagnoses of pediatric patients with ADHD and the administration of MPH to treat the symptoms have increased in prevalence in recent years. A 2005 study by El-Zein et al. reported statistically significant increases in cytogenetic anomalies including chromosomal aberrations (CA), micronuclei (MN) and sister chromatid exchanges (SCEs) in peripheral blood lymphocytes cultured from pediatric patients treated for 3 months with MPH. These findings led to widespread concern regarding the potential for genotoxic risks associated with prolonged administration of MPH. The study described in the present paper was designed to repeat the El-Zein effort with a much larger sample size. The subjects ($N = 109$) were randomized into two groups: one treated with MPH as well as behavior therapy, the other was a control group that received behavior therapy only. We evaluated CAs, MN, and SCEs in peripheral blood lymphocytes in samples obtained prior to therapy and after 3 months of treatment with MPH. The data were analyzed using a Poisson regression model with a generalized estimating equation method adjusted for several covariates including time, treatment-by-time interaction, sex, and age group. The log_e rate ratios of the MPH plus behavior therapy and behavior therapy groups were compared. The frequencies of CAs, MN, and SCEs were not increased in the MPH plus behavior therapy group when compared to the behavior therapy group only ($p = 0.53, 0.28, 0.81$, respectively). These results provide evidence in a large cohort that MPH does not induce cytogenetic anomalies in children, in contrast to the findings of the El-Zein study.

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1. Introduction

Methylphenidate (MPH) has been used for over 50 years to treat patients with attention deficit/hyperactivity disorder (ADHD). In recent years the diagnosis of ADHD and the use of prescription medications to treat this condition have increased substantially. Between 1990 and 1995 the number of patients in the United States aged 5–18 years diagnosed with ADHD increased 2.5-fold from 947,208 to 2,357,833. The number of patients prescribed stimulant pharmacotherapy increased 2.9-fold, and the number of ADHD patients prescribed MPH increased 2.6-fold [1].

In 2005, El-Zein et al. reported highly significant increases in chromosomal aberrations (CAs), micronuclei (MN), and sister chromatid exchanges (SCEs) in cultured peripheral blood lymphocytes from 12 children aged 6–12 years who were treated for 3 months with 20–54 mg/day MPH [2]. A short time later, several problems with the paper and the study design were pointed out [3]. A subsequent site visit to the El-Zein laboratory by experts from the NIH and the US FDA clarified some of the problems [4] including poor

quality of the slide preparations, low mitotic indices, and faded staining. Nevertheless, the findings of El-Zein et al. raised concerns in the health care community because elevated frequencies of CAs and MN have been associated with significantly increased risks of cancer [5,6].

Subsequently, several studies using humans and mice were conducted to gain further insights into the potential genotoxicity of MPH. None of these studies reported increases in genetic damage [7–9]. A study using Rhesus monkeys (*Macaca mulatta*) treated with MPH over a 20-month period was negative for increases in micronucleated reticulocytes, hypoxanthine phosphoribosyl transferase (Hprt) mutants and chromosome translocations measured by fluorescence *in situ* hybridization whole chromosome painting [10]. However, there was a weak positive effect observed with the Comet assay in neural tissue of MPH-exposed rats [11], a finding attributed to reactive oxygen species formed during metabolism of MPH in the rat brain tissues [12].

The study presented here was designed to replicate the work of El-Zein et al. [2] with a larger sample size to determine whether administration of extended-release Ritalin® LA (MPH) in treatment-naïve children with ADHD affects the frequency of cytogenetic anomalies. No significant increases in the frequencies of CAs, MN, and SCEs were observed between baseline sampling and the 3-month sampling time in either the MPH plus behavior therapy group or in the behavior therapy only group.

2. Materials and methods

2.1. Study design

This was an open-label, behavior-treatment-controlled evaluation of extended-release MPH. There was a screening period of up to 28 days (Day –29 to Day –1), a baseline visit, and a 3-month treatment period with clinic visits. At baseline, all eligible patients were randomized to one of the two parallel treatment groups: MPH plus behavior therapy, and behavior therapy only. Children of both genders were diagnosed with ADHD of any type according to the Diagnostic and Statistical Manual of Mental Disorders-IV and who had age-appropriate cognitive functioning were enrolled. Randomization was stratified by age group (6–8 years and 9–12 years), and by center. Blood samples were obtained from all participating patients for evaluation of cytogenetic damage by blinded slide readers at baseline and after 3 months of treatment.

Study participants were excluded if they had: previous exposure to MPH or any amphetamine-based medication, a positive urine drug screen, an abnormality in the screening assessment (physical exam, vital signs, laboratory tests) that was judged to be clinically significant, a cardiac abnormality, a history of seizures or schizophrenia, or a current diagnosis of mood disorder or anxiety disorder.

Study participants received MPH starting with 10 mg/day, which could be increased weekly in intervals of 10 mg/day to a maximum of 60 mg/day for a total treatment duration of 3 months (84 days). MPH was administered to achieve the desired clinical effect with minimum or no side effects. While the maximum doses differed, 30–40 mg/day was typical for the majority of the patients.

All patients received behavioral therapy based on the program of Disruptive Behavior Intervention that was designed both for the children and the parents. Clinicians trained in behavioral therapy for ADHD implemented this program. In general, the therapy consisted of two parts: Phase I was designed to target key symptoms of the disease (non-compliance, off-tasks, impulse behavior) and Phase II sessions focused on organizational skills, family communication, and discipline. A maximum of 17 sessions were scheduled individually and were evenly spread over the course of the study.

This study was approved by the ethics committees for all 17 study centers, which were located in the United States. A written informed consent by parent or legal guardian was obtained for all children before participation in the study. Patients were recruited between November 2006 and March 2008 from those participating study centers based on the pre-defined inclusion and exclusion criteria described above.

2.2. Blood shipping and tissue culture

Blood samples were obtained in heparinized vacutainers and shipped from the clinical sites overnight to the cytogenetics laboratory at Wayne State University. Cold packs were included in the shipping containers to maintain a temperature of approximately 4 °C while in transit. Upon receipt, 800 µl of blood was cultured in 10 ml media at 37 °C with 5% CO₂ for 48 h (CA) or 72 h (SCEs and MN) with 0.1 µg/ml Colcemid (final concentration, Invitrogen) added to the CA and SCE cultures 4 h prior to harvest. For the MN cultures, 6 µg/ml (final concentration) Cytochalasin

B (Sigma) dissolved in dimethyl sulfoxide (Fisher) was added 28 h prior to harvest. All culture media consisted of RPMI 1640 (Invitrogen) supplemented with 100 U/ml penicillin G, 100 µg/ml streptomycin (Invitrogen), 2 mM L-Glutamine (Invitrogen), 1% phytohemagglutinin (Invitrogen) and 15% fetal bovine serum (FBS, Atlanta Biologicals). Cultures for SCEs and CAs were further supplemented with 100 µM and 25 µM bromodeoxyuridine (Sigma), respectively.

2.3. Cytogenetic slide preparation

The CA and SCE cultures were harvested by centrifugation at 1400 rpm for 5 min to obtain pellets. The cells were then suspended in 8 ml of pre-warmed 75 mM KCl hypotonic solution and incubated for 30 min at 37 °C. Cells were fixed four times in methanol: acetic acid (3:1), then dropped onto microscope slides suspended on a rack located 1–2 cm above a 70 °C H₂O water bath and left until the fixative evaporated. The MN slides were prepared by gently mixing the cultures to reduce cellular clumping. A 200–300 µl aliquot of cell culture was then placed in a filter adapter (Statspin) containing a microscope slide and applied to the slide by centrifugation in a cytofuge (Statspin) at 1300 rpm for 4 min. The cells on the slides were fixed in absolute methanol for 10 min and allowed to air dry. The slides for all three endpoints were stored in slide boxes at ambient room temperature until stained.

2.4. Slide staining

The MN slides were stained in 10% Giemsa in Sorenson's buffer (67 mM Na₂HPO₄, 67 mM KH₂PO₄, pH 6.8), then briefly rinsed in dH₂O and air dried. Slides for the CA and SCE endpoints were incubated in 0.005% Hoechst 33258 in Sorenson's buffer for 10 min, rinsed and air dried, then placed in a staining tray and overlaid with Sorenson's buffer and exposed to 400–600 Å light for 15 min in a Spectrolinker XL-1000 UV crosslinker (Spectronics Corporation). The slides were rinsed briefly in dH₂O then air dried and stained in 10% Giemsa in Sorenson's buffer for 10 min. After staining the slides were briefly rinsed in dH₂O and allowed to air dry. All slides were mounted with Permount (Fisher) and a cover slip.

2.5. Endpoint scoring

Samples were coded at the clinical sites such that the cytogenetics laboratory was blinded with respect to each patient's treatment group. Furthermore, the slide readers were additionally blinded to the study design and specifically were not informed of the sequential nature of the blood sampling.

The number of cells scored for each cytogenetic endpoint was as follows: CA—200 first division cells; SCEs—50 second division cells; MN—2000 cytokinesis blocked binucleated cells. With rare exceptions ($N=3$ instances, when only half the desired number of cells could be scored for a sample), exactly half the cells for each sample and endpoint were scored by one slide reader and the other half were scored by a different reader. For MN and SCEs, the two halves of the scoring results for each donor and time point were compared and in the rare cases of substantial discrepancies, the slides were re-scored. For CAs, the aberrations scored included chromosome breaks and exchanges, and chromatid breaks and exchanges. Chromosome gaps and chromatid gaps were also recorded but these were not considered to be aberrations and were excluded from the statistical analyses. Every cell with a putative exchange, break, or gap, was checked through the microscope for accuracy by one individual (JDT). All scoring was performed on Nikon Eclipse E200 bright field microscopes at 1000× magnification with an oil immersion lens.

2.6. Statistical analyses

Initially, a statistical power analysis determined that a sample size of 38 subjects per treatment group was necessary to achieve at least 98% power for detecting a doubling in the Day 84 vs. Baseline rate ratios for all three cytogenetic endpoints. An estimated rate of 30% non-evaluable subjects led to a total of sample size of 54 patients per treatment group to be recruited.

The primary variables, i.e. the number of CAs per 100 cells and the number of MN per 1000 cells, were not normally distributed. Poisson regression was used to model these two endpoints with person correlation accounted for by fitting an exchangeable covariance structure through generalized estimating equations methodology. The factors in the model included time, treatment-by-time interaction, sex and age group. The main effect of treatment was not included as a factor since it was assumed that there was no treatment difference at baseline due to randomization.

The average number of SCEs per cell was not normally distributed. A log-linear model was fitted for this endpoint. The same factors and covariance structure as used in Poisson regression were included in this model.

The Day 84 vs. Baseline rate ratios for CAs, MN and SCEs were estimated from the Poisson regression or log-linear models. By comparing each rate ratio to one, we determined which direction the rate changed at the end of treatment compared to the baseline values. The between-treatment-group ratios of the Day 84 vs. Baseline ratios were also estimated from the above models. If the estimated Day 84 vs. Baseline rate ratios were larger than one for both the Ritalin-plus-behavior group and the behavior-only group, the between-treatment-group ratio was used to determine which group had the largest fold-increase.

Table 1
Patient disposition by treatment—all randomized patients.

	MPH + behavior N (%)	Behavior N (%)	Total N (%)
Screened			142
Randomized	53 (100.0%)	56 (100.0%)	109 (100.0%)
Ages 6–9 years	40 (75.5%)	39 (69.6%)	79 (72.5%)
Ages 10–12 years	13 (24.5%)	17 (30.3%)	30 (27.5%)
Completed	38 (71.7%)	39 (69.6%)	77 (70.6%)
Discontinued	15 (28.3%)	17 (30.4%)	32 (29.4%)
Main cause of discontinuation			
Abnormal test procedure result(s)	1 (1.9%)	0 (0.0%)	1 (0.9%)
Unsatisfactory therapeutic effect	0 (0.0%)	1 (1.8%)	1 (0.9%)
Protocol deviation	6 (11.3%)	2 (3.6%)	8 (7.3%)
Patient withdrew consent	3 (5.7%)	8 (14.3%)	11 (10.1%)
Lost to follow-up	0 (0.0%)	6 (10.7%)	6 (5.5%)
Administrative problems	5 (9.4%)	0 (0.0%)	5 (4.6%)

The statistical analyses of CAs and MN were based on patients who were randomized and provided cytogenetic data for at least one of these two endpoints at baseline and at the 3-month evaluation. The statistical analyses on SCEs were based on patients who were randomized and provided at least one post-baseline efficacy assessment.

3. Results

The patient disposition by treatment is shown in Table 1. Of the 109 randomized patients, 104 were included in the study population. Among the randomized patients, nearly three out of four subjects were aged 6–9 years, the rest were 10–12 years old. Of the 109 randomized patients, 77 (70.6%) completed the study. The primary reasons patients discontinued the study were withdrawal of consent (10.1%), deviations from the protocol (7.3%), loss to follow-up (5.5%), and administrative problems (4.6%).

The demographic and baseline characteristics by treatment at the beginning of the study are shown in Table 2. The mean and median ages, gender distribution, and race were not substantially different between the two treatment groups. Table 3 shows the demographic information for subjects by treatment group at Day 84 of the study. No substantive changes occurred between Day 1 and Day 84 for any of the demographic characteristics.

Tables 4–6 provide the frequencies of CAs, MN, and SCEs, respectively, by age group and sampling time. There were no significant differences between the two treatment groups for any of the endpoints, for either age group, or for all children regardless of age.

The CA frequencies for the treatment groups at baseline and Day 84 treatment completion are shown in Fig. 1. Based on the Poisson

Table 2
Demographic and baseline characteristics by treatment, beginning of the study (Day 1).

	MPH + behavior	Behavior	Total
Age (years)			
N	52	52	104
Mean ± SD	8.3 ± 1.75	8.5 ± 1.91	8.4 ± 1.83
Median	8.0	8.0	8.0
Range (Min, Max)	6, 12	6, 12	6, 12
Sex N (%)			
Male	31 (59.6%)	35 (67.3%)	66 (63.5%)
Female	21 (40.4%)	17 (32.7%)	38 (36.5%)
Race N (%)			
Caucasian	37 (71.2%)	39 (75.0%)	76 (73.1%)
Black	14 (26.9%)	11 (21.2%)	25 (24.0%)
Other	1 (1.9%)	2 (3.8%)	3 (2.9%)

Table 3
Demographic characteristics by treatment, Day 84 (Week 12) of the study.

	MPH + behavior	Behavior	Total
Age (years)			
N	47	45	92
Mean ± SD	8.1 ± 1.64	8.5 ± 1.96	8.3 ± 1.80
Median	8.0	8.0	8.0
Range (Min, Max)	6, 12	6, 12	6, 12
Sex N (%)			
Male	28 (59.6%)	30 (66.7%)	58 (63.0%)
Female	19 (40.4%)	15 (33.3%)	34 (37.0%)
Race N (%)			
Caucasian	35 (74.5%)	35 (77.8%)	70 (76.1%)
Black	11 (23.4%)	8 (17.8%)	19 (20.7%)
Other	1 (2.1%)	2 (4.4%)	3 (3.3%)

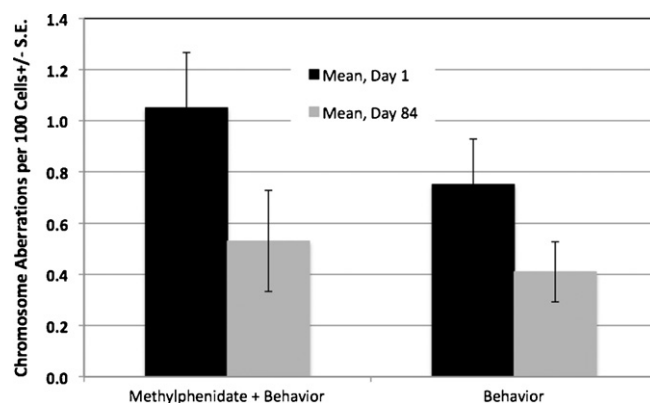


Fig. 1. Structural chromosomal aberration frequencies by treatment group (MPH plus behavior therapy vs. behavior therapy alone) and time (Day 1 vs. Day 84). Vertical bars indicate the standard errors of the means. There were 33 and 32 subjects, respectively, in the MPH plus behavior therapy group and in the behavior therapy group alone.

model, the log_e rate ratios of CAs observed at Day 84 vs. baseline for MPH plus behavior therapy was 0.60 (40% decrease) and for behavior therapy only control was 0.44 (56% decrease). The ratio between the two treatment groups was 0.60/0.44 = 1.34, which is not statistically significant (*p* = 0.53). The frequencies of MN for the treatment groups at baseline and Day 84 are shown in Fig. 2. The log_e rate ratios of MN observed at Day 84 vs. baseline for MPH plus behavior therapy was 0.63 (37% decrease) and for behavior therapy only was 0.74 (26% decrease). The ratio between the two treatment groups was 0.63/0.74 = 0.85 and is not statistically significant (*p* = 0.28). The SCE frequencies for the treatment groups at baseline and Day 84 are

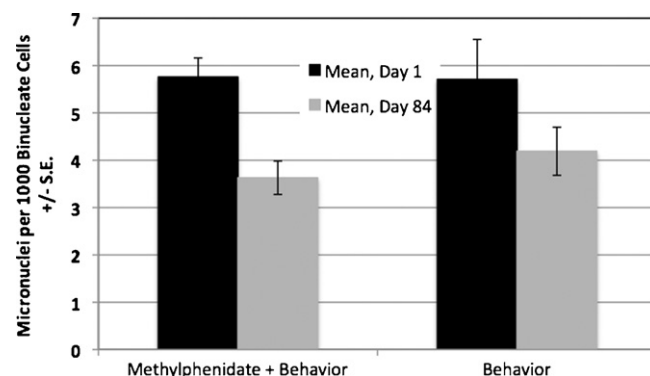


Fig. 2. Micronucleus frequencies by treatment group (MPH plus behavior therapy vs. behavior therapy alone) and time (Day 1 vs. Day 84). Vertical bars indicate the standard errors of the means. There were 34 and 29 subjects, respectively, in the MPH plus behavior therapy group and in the behavior therapy group alone.

Table 4
Chromosomal aberration frequencies per 100 cells, excluding gaps, by age group and sampling time.

	MPH + behavior	Behavior
Age group 6–9		
Baseline (Day 1)		
N	29	21
Mean	1.00	0.43
SD	1.22	0.68
25%	0.5	0.0
Median	0.50	0.00
75%	1.0	0.5
Range (Min, Max)	0.0, 5.5	0.0, 2.0
Day 84 (Week 12)		
N	27	21
Mean	0.59	0.50
SD	1.23	0.79
25%	0.0	0.0
Median	0.00	0.50
75%	0.5	0.5
Range (Min, Max)	0.0, 6.0	0.0, 3.5
Change from baseline to Day 84		
N	27	20
Mean	−0.43	0.05
SD	1.77	0.74
25%	−1.0	−0.3
Median	−0.50	0.00
75%	0.0	0.5
Range (Min, Max)	−5.0, 5.5	−1.5, 1.5
Age group 10–12		
Baseline (Day 1)		
N	6	12
Mean	1.17	1.29
SD	1.37	1.23
25%	0.0	0.5
Median	0.75	0.75
75%	2.5	2.3
Range (Min, Max)	0.0, 3.0	0.0, 4.0
Day 84 (Week 12)		
N	7	13
Mean	0.21	0.27
SD	0.39	0.33
25%	0.0	0.0
Median	0.00	0.00
75%	0.5	0.5
Range (Min, Max)	0.0, 1.0	0.0, 1.0
Change from baseline to Day 84		
N	6	12
Mean	−0.92	−1.00
SD	1.56	1.38
25%	−2.5	−2.3
Median	−0.50	−0.50
75%	0.0	0.0
Range (Min, Max)	−3.0, 1.0	−3.5, 1.0
All ages		
Baseline (Day 1)		
N	35	33
Mean	1.03	0.74
SD	1.22	0.99
25%	0.0	0.0
Median	0.50	0.50
75%	1.5	1.0
Range (Min, Max)	0.0, 5.5	0.0, 4.0
Day 84 (Week 12)		
N	34	34
Mean	0.51	0.41
SD	1.12	0.66
25%	0.0	0.0
Median	0.00	0.25
75%	0.5	0.5
Range (Min, Max)	0.0, 6.0	0.0, 3.5
Change from baseline to Day 84		
N	33	32
Mean	−0.52	−0.34
SD	1.72	1.13
25%	−1.0	−0.8
Median	−0.50	0.00
75%	0.0	0.3
Range (Min, Max)	−5.0, 5.5	−3.5, 1.5

Table 5
Micronucleus frequencies per 1000 binucleated cells by age group and sampling time.

	MPH + behavior	Behavior
Age group 6–9		
Baseline (Day 1)		
N	29	21
Mean	5.64	6.88
SD	2.36	5.63
25%	4.0	3.0
Median	5.50	4.50
75%	7.0	8.0
Range (Min, Max)	1.0, 10.5	1.5, 25.0
Day 84 (Week 12)		
N	28	20
Mean	3.73	4.60
SD	2.06	3.14
25%	2.0	2.3
Median	3.75	4.00
75%	5.3	6.3
Range (Min, Max)	0.5, 9.0	0.5, 14.5
Change from baseline to Day 84		
N	28	19
Mean	−2.02	−2.05
SD	2.79	6.46
25%	−3.8	−5.5
Median	−2.00	−0.50
75%	0.0	3.0
Range (Min, Max)	−8.0, 4.5	−23.5, 5.5
Age group 10–12		
Baseline (Day 1)		
N	6	11
Mean	5.83	4.00
SD	2.60	1.94
25%	4.0	2.5
Median	5.00	3.00
75%	7.0	6.0
Range (Min, Max)	3.5, 10.5	2.0, 7.5
Day 84 (Week 12)		
N	7	12
Mean	3.50	4.00
SD	2.14	1.86
25%	1.0	2.5
Median	3.50	4.00
75%	5.5	5.0
Range (Min, Max)	0.5, 5.5	1.5, 7.5
Change from baseline to Day 84		
N	6	10
Mean	−2.67	−0.50
SD	3.64	2.84
25%	−3.0	−3.5
Median	−2.00	0.50
75%	−0.5	2.0
Range (Min, Max)	−9.5, 1.0	−4.5, 2.5
All ages		
Baseline (Day 1)		
N	35	32
Mean	5.67	5.89
SD	2.37	4.86
25%	4.0	2.5
Median	5.50	4.25
75%	7.0	8.0
Range (Min, Max)	1.0, 10.5	1.5, 25.0
Day 84 (Week 12)		
N	35	32
Mean	3.69	4.38
SD	2.05	2.71
25%	2.0	2.3
Median	3.50	4.00
75%	5.5	5.8
Range (Min, Max)	0.5, 9.0	0.5, 14.5
Change from baseline to Day 84		
N	34	29
Mean	−2.13	−1.52
SD	2.91	5.47
25%	−3.5	−3.5
Median	−2.00	−0.50
75%	0.0	2.0
Range (Min, Max)	−9.5, 4.5	−23.5, 5.5

Table 6
SCE frequencies by age group and sampling time.

	MPH + behavior	Behavior
Age group 6–9		
Baseline (Day 1)		
N	22	17
Mean	7.70	7.49
SD	0.71	1.20
25%	7.28	6.80
Median	7.68	6.92
75%	8.12	8.22
Range (Min, Max)	6.44, 9.22	5.84, 9.48
Day 84 (Week 12)		
N	24	12
Mean	7.22	6.78
SD	1.04	0.90
25%	6.55	5.93
Median	7.30	7.05
75%	7.74	7.46
Range (Min, Max)	5.18, 9.66	5.34, 7.84
Change from baseline to Day 84		
N	17	8
Mean	-0.45	-0.08
SD	1.25	1.46
25%	-1.28	-1.23
Median	-0.48	-0.31
75%	0.14	1.24
Range (Min, Max)	-2.56, 2.08	-1.96, 1.92
Age group 10–12		
Baseline (Day 1)		
N	5	11
Mean	8.65	7.49
SD	1.56	1.29
25%	7.44	6.48
Median	7.82	7.22
75%	10.28	8.42
Range (Min, Max)	7.30, 10.40	5.46, 9.72
Day 84 (Week 12)		
N	5	7
Mean	6.85	7.05
SD	1.20	1.04
25%	6.20	6.52
Median	6.46	6.96
75%	6.48	8.10
Range (Min, Max)	6.12, 8.98	5.32, 8.34
Change from baseline to Day 84		
N	3	6
Mean	-1.41	-0.43
SD	2.73	1.51
25%	-4.28	-1.90
Median	-1.10	-0.45
75%	1.16	0.52
Range (Min, Max)	-4.28, 1.16	-2.16, 1.86
All ages		
Baseline (Day 1)		
N	27	28
Mean	7.88	7.49
SD	0.96	1.21
25%	7.30	6.77
Median	7.78	7.11
75%	8.32	8.38
Range (Min, Max)	6.44, 10.40	5.46, 9.72
Day 84 (Week 12)		
N	29	19
Mean	7.16	6.88
SD	1.06	0.93
25%	6.48	6.26
Median	7.22	6.96
75%	7.72	7.52
Range (Min, Max)	5.18, 9.66	5.32, 8.34
Change from baseline to Day 84		
N	20	14
Mean	-0.59	-0.23
SD	1.49	1.43
25%	-1.50	-1.88
Median	-0.55	-0.40
75%	0.35	1.02
Range (Min, Max)	-4.28, 2.08	-2.16, 1.92

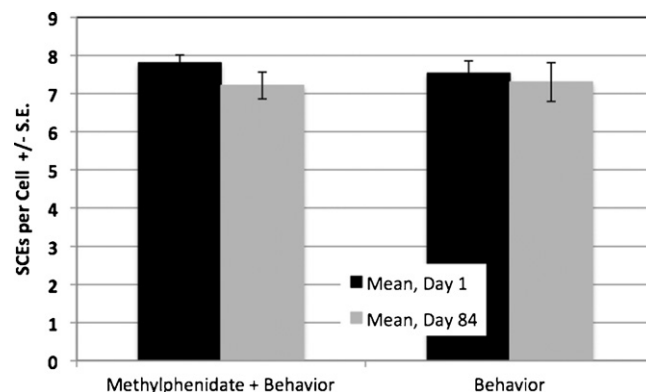


Fig. 3. Sister chromatid exchange frequencies by treatment group (MPH plus behavior therapy vs. behavior therapy alone) and time (Day 1 vs. Day 84). Vertical bars indicate the standard errors of the means. There were 20 and 14 subjects, respectively, in the MPH plus behavior therapy group and in the behavior therapy group alone.

shown in Fig. 3. Based on the log-normal model, the \log_e rate ratios of SCEs observed at Day 84 vs. baseline for MPH plus behavior therapy was 0.94 (6% decrease) and for behavior therapy only was 0.95 (5% decrease). The ratio between the two treatment groups was $0.94/0.95 = 0.99$, which is not statistically significant ($p = 0.81$). The 95% confidence intervals for the rate ratio of Day 84 to baseline all included 1, except the following ratios had an upper limit below 1: number of CAs in the behavior-only group (0.44 {0.24, 0.83}), and the number of MN in the MPH-treated group (0.63 {0.50, 0.79}) and the untreated group (0.74 {0.55, 0.99}).

4. Discussion

None of the three cytogenetic endpoints evaluated in this study, i.e. structural chromosomal aberrations, micronuclei, and sister chromatid exchanges, showed a statistically significant increase following 84 days of methylphenidate therapy, either in the MPH plus behavior therapy group or in the group that received behavior therapy alone. These results stand in marked contrast to the findings of El-Zein et al. [2] who reported significant increases in all three endpoints with a similar study design but with a smaller sample size and without a behavior therapy only control group. Furthermore, no significant difference was observed in the rate ratio between pretherapy (baseline) and Day 84 values in the behavior-only group for CAs, MN, and SCEs ($p = 0.53, 0.28$ and 0.81 respectively).

The pediatric patients in this study were treated with MPH at doses that began at 10 mg/day and were evaluated weekly; if deemed appropriate, the dose was increased by 10 mg/day with a maximal dose of 60 mg/day. Each patient was treated to achieve the desired behavioral outcome, so both the daily dose administered and the total accumulated dose over the course of this study differed according to the individualized needs of the patients.

Our results are consistent with the findings of others. Manjanatha et al. [7] evaluated mice treated with MPH and saw no significant increase in either the Hprt mutant frequency or the MN frequency in the treated animals. Andreazza et al. [11] evaluated young and adult rats and used the Comet assay to measure early DNA damage in cells from hippocampus, striatum and peripheral blood, and used the Cytochalasin B-block method to evaluate MN frequencies in peripheral blood. MPH led to an increase in DNA damage as measured by the Comet assay, which was more pronounced with chronic treatment and in the striatum compared to the hippocampus. However, neither acute nor chronic MPH treatment increased the frequency of MN in young or in adult rats. In a study of juvenile Rhesus macaques, Morris et al. [10] showed that chronic MPH exposure at doses that were observed to

influence behavior did not result in an increase in the frequency of chromosome translocations, micronucleated reticulocytes, or Hprt mutations. Suter et al. [8] conducted a chromosomal aberration study in cultured human peripheral lymphocytes and showed that MPH in concentrations up to 10 mM did not induce structural or numerical chromosome abnormalities. In a prospective study, Walitza et al. [9] evaluated the peripheral lymphocytes of children and saw no significant change in MN frequencies over a 6-month period. Witt et al. [13] evaluated 25 subjects aged 6–12 years who completed a 3-month regimen of MPH. Evaluations of CAs, MN, and SCEs revealed no significant treatment-related increases in any of these three endpoints. Stopper et al. [14] evaluated the frequency of MN in a prospective study of 38 children before and up to 6 months after treatment with MPH. No significant change was observed in these frequencies. Oestreicher et al. [15] examined the risk of cancer among 35,400 MPH users, who took this medication before age 20, and found no moderate or strong association between MPH use and cancer risk in children.

Other than the initial report by El Zein et al. [2] the available cytogenetic data are consistent with the observations reported here that MPH does not induce significant increases in CAs, MN, or SCEs in pediatric subjects. Evaluations of other mutational endpoints are consistent with the human and animal cytogenetic data. Taken together, these findings show a high degree of consistency and support the notion that MPH does not induce chromosomal alterations or other types of genetic damage in children treated for ADHD.

Conflict of interest

The authors declare a conflict of interest. This work was funded by Novartis Pharmaceuticals Corporation, and some of the authors are employed by this company.

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