Title

Change in Plasma Cytokine Levels During Risperidone Treatment in Children with Autism

Journal

Journal of Child and Adolescent Psychopharmacology, 24(10)

ISSN

1044-5463

Authors

Choi, Jae Eun

Widjaja, Felicia

Careaga, Milo

et al.

Publication Date

2014-12-01

DOI

10.1089/cap.2013.0108

Peer reviewed

Change in Plasma Cytokine Levels During Risperidone Treatment in Children with Autism

Author Information

[1]Jae Eun Choi, BA; [2]Felicia Widjaja, MPH; [3]Milo Careaga, BS; [4]Stephen Bent, MD; [3]Paul Ashwood, PhD; [2]Robert L. Hendren, DO

[1]School of Medicine, University of California, San Diego; [2]Department of Psychiatry, University of California, San Francisco; [3]MIND Institute, University of California, Davis; [4]Department of Internal Medicine, University of California, San Francisco
Funding: This study was supported by R21MH080026 (RLH) from NIMH.

Address correspondence to:
Robert L. Hendren, DO
University of California, San Francisco
401 Parnassus Ave
San Francisco, CA 94143-0984
Robert.Hendren@ucsf.edu

Abstract

Background: Atypical antipsychotics decrease irritability in autism. They also affect the cytokine network. Psychological stress, depression and possibly ASD are shown to stimulate the production of pro-inflammatory cytokines. We sought to determine if risperidone treatment led to changes in cytokine levels.

Methods: 45 subjects from an open-label study of risperidone treatment of children with ASD ages 4-18 years had analysis of 27 different cytokines at baseline and after 8-weeks of treatment using multiplex assays (Millipore) and read on the Luminex 100^{TM} platform. We examined changes in each of the cytokine levels in the entire group, and also compared changes in cytokines in responders vs. non-responders.

Results: After 8 weeks of risperidone treatment, two of the 27 cytokines showed statistically significant decreases in median levels: Eotaxin (p=.0003) and MCP1 (p=.0024). Six of the 48 children met two criteria for responders to risperidone, and one cytokine level (IL-5) had a statistically significantly greater change (increase) in the responder compared to the non-responder group.

Conclusion: Two cytokines, Eotaxin and MCP1, which have previously been identified as abnormally elevated in children with autism, decreased during treatment with risperidone. This suggests a possible mechanism of action of risperidone treatment and a balancing of the immune system in affected children.

Introduction

Atypical antipsychotics, such as risperidone and aripiprazole, are shown to decrease behavioral disturbances, such as irritability, aggression and anxiety among children with autism (Myers et al. 2007; McCracken et al. 2002). There are a growing number of studies reporting that psychological stress, psychosis or depression can directly stimulate the production of pro-inflammatory cytokines and that treatment with antipsychotic drugs affects the cytokine network (Na et al. 2012; Himmerich et al. 2011).

To date, reports regarding the effects of antipsychotics on cytokine levels are inconsistent and no antipsychotic has been shown to have consistent anti-inflammatory action (Drzyzga et al. 2006). Multiple studies of schizophrenia support the anti-inflammatory profile of risperidone through an increase in T_H2-type cytokines and a shift towards T_H2 responses (Chen, 2011; Teixeira, 2007). There are a limited number of studies finding an association between treatment and immune response in ASD (Ashwood et al. 2006). Previous studies investigating inflammatory abnormalities related to ASD have yielded conflicting and inconclusive results (Onore et al. 2012). However, studies do support a pro-inflammatory and T_H1 skewed profile in autistic subjects in CD4+ cells and an association of these changes with more severe behavioral symptoms (Ashwood, 2011a).

Recently, a study reported that clinical improvement in children with ASD following 8 weeks of treatment with risperidone was not associated with changes in plasma levels of cytokines (Tobiasova et al. 2011). In the study we report here, our hypotheses were that risperidone will (1) demonstrate anti-inflammatory properties as shown through a decrease in pro-inflammatory and T_H1 cytokines and an increase in T_H2 cytokines, and that (2) these changes will be associated with a favorable treatment response.

Methods

Study Design: The primary goal of this project was to determine whether cytokine levels change during risperidone treatment in children with autism. The UC Davis Institutional Review Board approved all study procedures prior to initiating the study. Informed consent was obtained from parents and assent was obtained from the child, when developmentally appropriate, before any study procedures were performed. This study is registered on clinicaltrials.gov (NCT00584701).

Subjects: Children ages 4-18 years were required to have a diagnosis of ASD as confirmed by consensus on the DSM IV diagnostic interview and the Autism Diagnostics Observation Schedule (ADOS), have an IQ > 55, and an Aberrant Behavior Checklist Irritability (ABC-I) subscale rating of ≥ 18. Subjects were excluded if they were on antipsychotics within 8 weeks of entry into the study. Subjects were allowed to continue on other medications or treatments begun two months prior to study enrollment. Subjects agreed to keep their current medications and treatments constant during the duration of the study and to abstain from beginning any new treatments. This study also excluded children with a diagnosis of bipolar disorder, schizophrenia, ASD of known genetic cause, seizures, metabolic disturbance or severe illness in the past year, as previously described (Lit et al. 2012).

Dosing Schedule

Subjects began with an initial dosage of 0.5 mg at bedtime for 4 days and were uptitrated to 1 mg at the same time for 4 additional days if the previous dose had been tolerated. If the behavioral disturbances continued, 0.5 mg was added to the daily dose in the morning as tolerated to a maximum daily total of 1.5 mg of risperidone over the duration of the study (Lit et al. 2012).

Statistical analysis

Using the same parameters as McCracken *et al.* (2002), subjects were defined as overall responders to treatment if they had a decrease in Aberrant Behavior Checklist-Irritability subscale (ABC-I) $\geq 25\%$ and a Clinical Global Impression-Improvement (CGI-I) rating of "very much improved" or "much improved." Cytokine analysis was performed using multiplex assays (Millipore) according to manufacturer's recommendation and read on the Luminex 100™ platform. The significance of the change in cytokine levels between the responder and non-responder groups were determined using nonparametric Mann-Whitney tests. Correlations were determined using Spearman's rank correlation coefficient. We did not adjust p-values for multiple testing since this is an exploratory analysis (Thompson, 1998).

Results

Data was collected on the plasma levels of 27 different cytokines of 45 subjects (mean age of 114.3 months, SD=52.6). The data included 35 males and 10 females with a mean IQ of 59.9 (SD=25.0) and mean ADOS communication and social interaction total of 16.6 (SD=6.6). Of the 45 subjects, 35 (78%) demonstrated at least a 25% decrease in ABC-I subscale score alone and 11 (24%) received a rating of "very much improved" or "much improved" on the CGI-I. A total of 6 subjects (13%) met both criteria and were identified as responders.

Overall: The mean ABC-I decreased from 24.8 (SD=6.7) to 11.9 (SD=6.1) after the treatment period (95% CI of change: -15.3, -10.4) ($p<.00001$). Two of the plasma cytokines showed statistically significant changes (decreases) after risperidone treatment: Eotaxin ($p=.0003$) and monocyte chemoattractant protein-1 (MCP1) ($p=.002$). These decreases were not significantly associated with change in %ABC-I scores. These data are shown in Table 1 and Figure 1 below.

Responder vs. Nonresponders: There were no significant difference in baseline characteristics, including age ($p=.92$), IQ ($p=.31$), ADOS score ($p=.32$), and gender ($p=0.12$) between the responder and non responder groups. One cytokine showed a greater change in the 6 responders on two measures compared to the non-responder group. The change in the median values of IL-5 ($p=.005$) was significantly higher in the overall responder group compared to non-responders. The mean value of the change in IL-5 increased in the responder group (mean=0.442, SD=.49) whereas it decreased in the non-responder group (mean= -0.888, SD=4.5).

Conclusion

There was a significant decrease in Eotaxin and MCP-1 levels following 8 weeks of risperidone treatment in children with ASD. This finding is in support of Ashwood *et al.* in 2011b that the production of MCP-1 and Eotaxin were significantly higher in children with ASD compared with typically developing children and children with developmental delays other than ASD. Their results are consistent with data from other studies that showed an increase in protein levels of MCP-1 and Eotaxin in brain specimens from individuals with ASD (Vargas et al. 2005) or for pro-inflammatory cytokines in the blood of ASD children (Ashwood et al. 2011c). Moreover, a

2005 study by Mundo *et al.*, suggested the role of a polymorphism of the MCP-1 gene (SCYA2) as a resistance factor during antipsychotic treatment of patients with schizophrenia, suggesting an interesting link between chemokine levels and responses to treatment.

Furthermore, the IL-5 increases could suggest that the profile is changing from a T_H1 to a T_H2-type cytokine and that could suggest benefit as described in previous studies demonstrating the cytokine modulating effects of antispsychotics, including risperidone, *in vitro* and *in vivo*. In $CD4^+$ T cells a shift towards T_H2 responses was associated with better cognitive scores in children with ASD (Ashwood et al. 2011c).

Clinical Significance:

Together, these data suggest that the immune system may be being balanced as a result of risperidone treatment. Inflammatory processes are thought to play a role in autism and decreases may benefit symptoms but replication with larger samples is necessary to determine if this is a direct effect.

Authors Disclosure

Jae Eun Choi, BA, Felicia Widjaja, MPH, Milo Careaga, BS, Stephen Bent, MD, Paul Ashwood, PhD have no institutional or corporate/commercial relationships to disclose.

Robert L. Hendren, D.O. received research grants from Forest Pharmaceuticals, Inc., Curemark, BioMarin Pharmaceutical, Roche, Shire, Autism Speaks, the Vitamin D Council and NIMH and is on Advisory Boards for BioMarin, Forest, Coronado Biosciences, BioZeus, and Janssen.

Table 1: Cytokines tested in 45 subjects treated with risperidone

n=45	**Pre-Treatment**	**Post-Treatment**		
	Mean (SD)	**Mean (SD)**	***p***	**rho (*p*)**
TGF B1 Well 1	13040.2 (8885.9)	16407.8 (1973.7)	*.09*	.02 (*.91*)
TGF B2 Well 2	11412.3 (8711.9)	12794.0 (1485.6)	*.27*	.07 (*.65*)
BDNF	1558.4 (292.0)	1256.5 (129.5)	*.86*	.03 (*.83*)
Eotaxin	81.9 (41.5)	65.8 (30.2)	***.0003****	-.17 (*.27*)
GCSF	43.6 (75.1)	43.8 (66.4)	*.48*	-.08 (*.63*)
GMCSF	84.7 (78.0)	82.4 (11.7)	*.60*	.02 (*.88*)
INFa2	83.1 (202.3)	75.1 (166.8)	*.55*	-.20 (*.20*)
INFg	14.1 (20.8)	12.5 (16.1)	*.26*	-.005(*.97*)
IL-1a	286.8 (256.4)	292.6 (226.6)	*.90*	.05 (*.76*)
IL-1b	2.9 (5.7)	2.5 (4.3)	*.17*	.02 (*.91*)
IL-2	4.1 (12.5)	2.9 (8.8)	*.51*	.02 (*.91*)
IL-5	2.5 (8.1)	1.8 (4.3)	*.73*	-.001(*.95*)
IL-6	5.9 (11.3)	5.9 (12.7)	*.31*	.25 (*.11*)
IL-7	27.1 (59.4)	25.8 (54.8)	*.66*	-.14 (*.35*)
IL-8	10.3 (17.6)	9.0 (14.2)	*.36*	-.01 (*.93*)
IL-10	5.0 (4.4)	5.3 (4.6)	*.50*	.20 (*.21*)
IL-12p40	84.4 (102.2)	79.4 (93.5)	*.08*	-.17 (*.28*)
IL-12p70	7.2 (12.0)	5.2 (7.9)	*.11*	.07 (*.66*)
IL-13	3.0 (9.8)	2.1 (6.7)	*.08*	.16 (*.30*)
IL-15	4.9 (11.6)	4.8 (13.0)	*.50*	-.12 (*.46*)
IL-17	10.4 (20.3)	8.5 (15.9)	*.19*	.10 (*.53*)
IP-10	452.7 (413.2)	444.8 (308.1)	*.95*	.06 (*.72*)
MCP-1	313.0 (143.0)	265.7 (80.4)	***.002****	.11 (*.48*)
MIP-1a	46.8 (46.5)	44.0 (39.4)	*.20*	.02 (*.92*)
MIP-1b	27.4 (27.3)	25.8 (21.6)	*.64*	.23 (*.15*)
TNFa	4.2 (1.6)	4.0 (1.5)	*.09*	.13 (*.42*)
TNFb	6.9 (17.6)	8.6 (25.4)	*.39*	-.01 (*.95*)

Table 1 Comparison of cytokine levels at pre and post-treatment: Mean cytokine values at baseline and after 8 weeks are shown. P-values were determined using non-parametric Mann-Whitney tests. Correlations were determined using Spearman's rho coefficient.

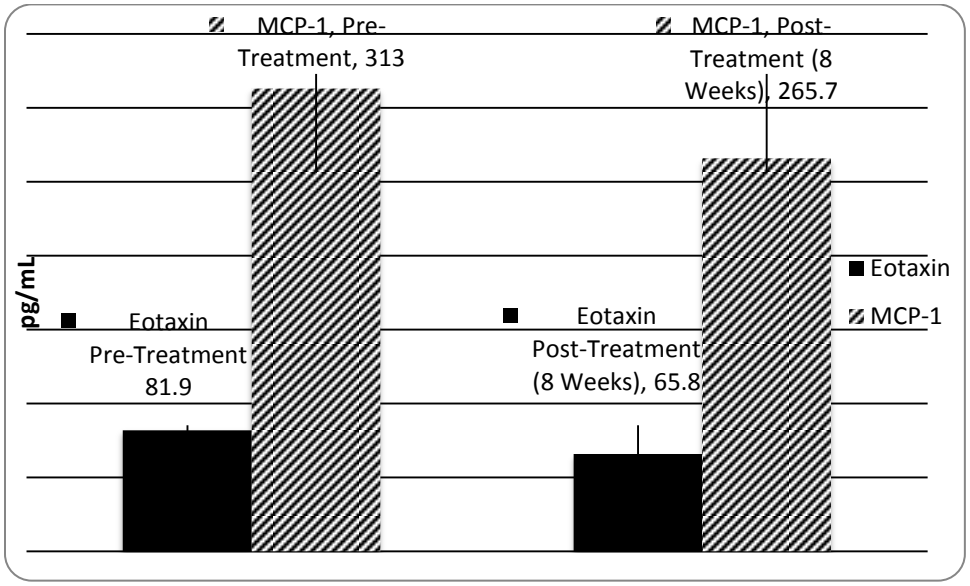

Figure 1: Mean values of Eotaxin and MCP-1 pre and post treatment (with standard deviations)

References:

Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah IN, Van de Water J. "Altered T cell responses in children with autism." *Brain Behavior, and Immunology*. 2011a Jul; *25*(5):840-9.

Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah IN, Van de Water J. "Associations of impaired behaviors with elevated plasma chemokines in autism spectrum disorders." *Journal of Neuroimmunology*. 2011b Mar; *232*(1-2):196-9.

Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah IN, Van de Water J. Altered T cell responses in children with autism. Brain Behav Immun. 2011c, Jul; *25*(5):840-9.

Ashwood, P, Wills, S and Van De Water, J. "The Immune Response in Autism: A New Frontier for Autism Research." *Journal of Leukocyte Biology*. 2006 Jul; *80*(1):1-15.

Bent S, Bertoglio K, Ashwood P, Bostrom A, Hendren RL. "A pilot randomized controlled trial of omega-3 fatty acids for autism spectrum disorder." *Journal of Autism Developmental Disorders*. 2011 May; *41*(5):545-54.

Chen ML, Tsai TC, Wang LK, Lin YY, Tsai YM, Lee MC, Tsai FM. "Risperidone modulates the cytokine and chemokine release of dendritic cells and induces TNF-α-directed cell apoptosis in neutrophils." *International Immunopharmacology*. 2012 Jan;*12*(1):197-204.

Drzyzga L, Obuchowicz E, Marcinowska A, Herman ZS. "Cytokines in schizoprenia and the effects of antipsychotic drugs." *Brain Behavavior, and Immunity*. 2006 Nov; *20*(6):532-45. Review.

Enstrom AM, Onore CE, Van de Water JA, Ashwood P. "Differential monocyte responses to TLR ligands in children with autism spectrum disorders." *Brain, Behavior, and Immunology*. 2010 Jan; *24*(1):64-71.

Gupta S, Aggarwal S, Rashanravan B, Lee T. "Th1- and Th2-like cytokines in CD4+ and CD8+ T cells in autism." *Journal of Neuroimmunology*. 1998 May 1; *85*(1):106-9.

Himmerich H, Schönherr J, Fulda S, Sheldrick AJ, Bauer K, Sack U. Impact of antipsychotics on cytokine production in-vitro. J Psychiatr Res. 2011 Oct; *45*(10):1358-65.

Lit L, Sharp FR, Bertoglio K, Stamova B, Ander BP, Sossong AD, Hendren RL. "Gene expression in blood is associated with risperidone response in children with autism spectrum disorders." *Pharmacogenomics Journal*. 2012 Oct; *12*(5):368-71.

McCracken JT, McGough J, Shah B, Cronin P, Hong D, Aman MG, Arnold LE, Lindsay R, Nash P, Hollway J, McDougle CJ, Posey D, Swiezy N, Kohn A, Scahill L, Martin A, Koenig K, Volkmar F, Carroll D, Lancor A, Tierney E, Ghuman J, Gonzalez NM, Grados M, Vitiello B, Ritz L, Davies M, Robinson J, McMahon D; Research Units on Pediatric Psychopharmacology Autism Network. "Risperidone in children with autism and serious behavioral problems." *New England Journal of Medicine*. 2002 Aug 1; *347*(5):314-21.

Mundo E, Altamura AC, Vismara S, Zanardini R, Bignotti S, Randazzo R, Montresor C, Gennarelli M. (2005). "MCP-1 gene (SCYA2) and schizophrenia: a case-control association study." *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics)*. 2005 Jan 5;*132B*(1):1-4.

Myers, SM and Johnson, CP. "Management of children with Autism Spectrum Disorders." *Pediatrics. 120*(5), 1162-182. [Epub 2007 Oct 29]

Na KS, Jung HY, Kim YK. (2012). The role of pro-inflammatory cytokines in the neuroinflammation and neurogenesis of schizophrenia. Prog Neuropsychopharmacol. Biol Psychiatry. [Epub ahead of print]

Onore C, Careaga M, Ashwood P. "The role of immune dysfunction in the pathophysiology of autism." *Brain, Behavior, and Immunology*. 2012 Mar; *26*(3):383-92.

Teixeira AL, Reis HJ, Nicolato R, Brito-Melo G, Correa H, Teixeira MM, Romano-Silva MA. "Increased serum levels of CCL11/eotaxin in schizophrenia." *Progress in Neuropsychopharmacoly and Biological Psychiatry*. 2008 Apr 1; *32*(3):710-4.

Thompson, J. R. (1998). Invited commentary: Re: ''Multiple comparisons and related issues in the interpretation of epidemiologic data''. *American Journal of Epidemiology*, *147*(9), 801–806.

Tobiasova Z, van der Lingen KH, Scahill L, Leckman JF, Zhang Y, Chae W, McCracken JT, McDougle CJ, Vitiello B, Tierney E, Aman MG, Arnold LE, Katsovich L, Hoekstra PJ, Volkmar F, Bothwell AL, Kawikova I. "Risperidone-related improvement of irritability in children with autism is not associated with changes in serum of epidermal growth factor and interleukin-13." *Journal of Child Adolescent Psychopharmacology*. 2011 Dec; *21*(6):555-64.

Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. "Neuroglial activation and neuroinflammation in the brain of patients with autism." *Annals of Neurology*. 2005 Jan; *57*(1):67-81.