Case Report

Beneficial Effects of Palmitoylethanolamide on Expressive Language, Cognition, and Behaviors in Autism: A Report of Two Cases

Nicola Antonucci, 1 Alessandra Cirillo, 2 and Dario Siniscalco 3,4,5

1 Biomedical Centre for Autism Research and Treatment, 70126 Bari, Italy
2 Institute of Biosciences and Bioresources, National Research Council of Italy, 80128 Naples, Italy
3 Department of Experimental Medicine, Second University of Naples, Via S. Maria di Costantinopoli, 80138 Naples, Italy
4 Centre for Autism-La Forza del Silenzio, 81036 Caserta, Italy
5 Cancellautismo-No Profit Association for Autism Care, 50132 Florence, Italy

Correspondence should be addressed to Dario Siniscalco; dariosin@uab.edu

Received 30 June 2015; Revised 15 September 2015; Accepted 16 September 2015

Academic Editor: Masaru Mimura

Copyright © 2015 Nicola Antonucci et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

1. Introduction

Autism spectrum disorders are defined by behavioral and language atypias. Growing body of evidence indicates inflammatory mediators may contribute to the condition. Palmitoylethanolamide (PEA) is naturally occurring and has been available as a nonprescription medical food supplement in Europe since 2008. PEA has been tested in thousands of human subjects without any noted significant side effects. Here we report the first cases of the administration of PEA to two children with autism.

Case Presentations

The first 13-year-old male child (Subject 1) presented with a total IgE of 572 IU/mL (nl < 200) and with low mature CD57+ natural killer cell counts (32 cells/μL; nl = 60–300 cells/μL) and with significant eczema and allergic stigmata. Expressive language, as measured by mean length of utterance, and overall autism severity as measured by the Childhood Autism Rating Scale, Second Edition, improved significantly. Atopic symptoms diminished. No side effects were reported. The second male child, age 15 (Subject 2), also displayed noticeable and rapid improvements in cognitive, behaviors, and sociability.

Conclusion

Currently, there is no definitive treatment for autism condition. Palmitoylethanolamide could be an effective treatment for autism syndrome. We propose appropriate double-blind clinical trials to further explore palmitoylethanolamide efficacy and safety.

1. Introduction

The link between autism spectrum disorders (ASDs) and inflammatory mechanisms both in utero and throughout child development has received extensive support from many sources. Masi et al. recently conducted an extensive review and meta-analysis of the published data on cytokine aberrations in ASDs and found significant evidence for increased interleukin- (IL-) 1beta (P < 0.001), IL-6 (P = 0.03), IL-8 (P = 0.04), interferon-gamma (P = 0.02), eotaxin (P = 0.01), and monocyte chemotactic protein-1 (P < 0.05) [1]. In the same study, they noted the deficit of a counter-regulatory cytokine, transforming growth factor-β1. In a detailed case-controlled study, Zerbo et al. also found elevations at birth of the fact that the chemokine monocyte chemotactic protein-1 (MCP-1) predicted future development of ASDs [2].

In a comprehensive review of sixty-seven studies, encompassing nearly 4000 children and adolescents, the evidence for a pro-inflammatory state was the strongest for ASDs when compared to major depressive disorder (MDD), bipolar disorder (BD), posttraumatic stress disorder (PTSD), obsessive-compulsive disorder (OCD), Tourette’s disorder (TD), attention-deficit/hyperactivity disorder (ADHD), and schizophrenia (SZ) [3].

Consistent with these noted inflammatory-state observations in the ASD population are the various immunological interventions published as potential autism therapies. Intravenous immunoglobulin, pentoxifylline, and transfer
factor were reviewed by Gupta wherein he noted a significant subpopulation responded to each intervention [4]. Of particular interest to this case was the use of pioglitazone, a thiazolidinedione-class agonist of the peroxisome proliferator activated receptor gamma (PPAR gamma), in an autism population [5]. The authors demonstrated pioglitazone significant reduced irritability, lethargy, stereotypy, and hyperactivity in the children with ASD.

However, pioglitazone significantly may increase the risk of bladder cancer and its use is therefore of limited value to ASDs [7]. The full evaluation of the other and various interventions of potential application of the ASD associated inflammatory state is beyond the scope of this brief report. However, in the search for safer alternatives possessing similar pharmacological effects, palmitoylethanolamide (PEA) emerges as a naturally occurring substance, which has been available as a nonprescription medical food supplement in Europe since 2008 [8]. Nobel laureate Aloe and colleagues initially defined its role as a regulator of mast cells, chronic pain, and inflammation in 1993 [9]. Of interest to ASDs and the large body of literature suggesting part of the chronic inflammatory state maintained in ASDs is mediated via the gastrointestinal system associated immune system [10,11] (including increased intestinal permeability) [12] is the new evidence describing the anti-inflammatory mechanisms of PEA in gut inflammation in a murine model [13]. The researchers found that exogenous palmitoylethanolamide (intraperitoneally and/or orally) decreased inflammation and improved intestinal permeability, while stimulating colonic cell proliferation and increasing colonic transient receptor potential vanilloid type-1 (TRPV1) and cannabinoid 1 receptor (CB1) expression. The observed anti-inflammatory effect of PEA was attenuated or abrogated by antagonists of the cannabinoid 2 receptor (CB2), the orphan G protein-coupled receptor-55 (GPR55). PPAR antagonists also attenuated the anti-inflammatory effect of PEA.

The potential CNS therapeutic effects of PEA were the subject of a recent and extensive review [14]. The authors note many desirable features of the molecule for chronic brain syndromes. Oral PEA administration has been tested in several thousand human subjects without any noted significant side effects [15,16]; however, data on its administration to children is limited primarily to treatment or prevention of viral-mediated upper respiratory tract illness [17,18]. ASDs are also felt to be mediated via complex interactions of microglial cells and mast cells in the central nervous system and macrophages and mast cells in the gastrointestinal system [19]. Recently the interaction between glial cells, mast cells, and the endocannabinoid system (ECS) was the subject of an extensive review [20]. The authors accumulated an abundance of literature supporting the role of mast cell-microglia cross talk in the development of many, if not all, chronic brain inflammatory disorders including autism.

2. Case Presentations

2.1. Consent. Written informed consent was obtained from the patient’s legal guardian(s) for treatment, publication of this case report, and all included data, in compliance with the Code of Ethical Principles for Medical Research Involving Human Subjects of the World Medical Association (Declaration of Helsinki). A copy of the written consent is available for review. All hematological testing was performed as a routine evaluation of the child’s immune disorders and was not ordered because of his autism diagnosis.

2.2. Subject 1. He is a 13-year-old autistic male with a well-documented history of significant atopic illnesses. He was originally diagnosed with autism by a child neurologist at age of 21 months following a significant regression in child development noted after a viral-like illness with associated high fevers (up to 45.5°C) at age of 15 months. Chronic urticaria, eczema, allergic rhinitis, and asthma have been treated with a variety of antihistamines, montelukast, and topical and systemic steroids since age of 2 years with only temporary beneficial effects. Serum food allergy testing revealed IgE-mediated responses to the following: corn, peanut, soybean, wheat, milk, rice, egg, and numerous other dietary proteins. Both skin prick testing and serum IgE inhalant allergy testing revealed similar patterns of significant reactions to most antigens tested including dust mites and nearly all grasses, trees, weeds, molds, and both domestic and farm animals. Both traditional desensitization with injectable antigens and sublingual immunotherapies have failed to reduce allergic stigmata and had no noticeable effects on the course of the child’s autism. Total serum IgE testing on numerous occasions has been significantly elevated and at the time PEA was introduced; it was 572 IU/mL (nl ≤ 200). Despite the high total levels of IgE and multiple IgE reactants, blood eosinophils have remained in the normal range throughout. Serum vitamin D OH25 levels remain deficient despite extensive efforts at oral supplementation, inferring a defect in absorption of fat-soluble nutrients. Serum vitamin D OH25 levels were at 21 ng/mL at the time PEA was started.

Cellular immune markers reflected specific abnormalities with deficient mature CD57+ natural killer (CD57+ NK) cell counts 32 cells/μL (nl = 60–300 cells/μL), with a total white count of 6.3 × 109/μL. However, the percentage of lymphocytes was high at 58%, while neutrophils were underrepresented at 33%, and absolute lymphocytes were also elevated at 3.5 × 109 cells/μL. All other obtained typical cell indices were in the normal range.

The Childhood Autism Rating Scale, Second Edition (CARS-2) [21], was administered as part of a routine evaluation of the child’s severity prior to a change that is his academic placement. Prior to PEA, CARS-2 scoring placed the child at 43.5 total (82nd percentile) with a verbal communication subscale of 3 out 4. A speech assessment was performed including a mean length of utterance (MLU) measurement. MLU is based on the linguistic concept of morphemes as the smallest component of speech [6]. Speech therapists use MLU as a routine assessment and, in this case, MLU was obtained prior to a change in school placement. MLU was observed to be 3.0 prior to PEA and this corresponded to an age equivalent of approximately 34–35 months (just under a 3-year-old level of speech).

Written informed consent was obtained from the patient’s legal guardian(s) for treatment, publication of this case report, and all included data, in compliance with the Code of Ethical Principles for Medical Research Involving Human Subjects of the World Medical Association (Declaration of Helsinki). A copy of the written consent is available for review. All hematological testing was performed as a routine evaluation of the child’s immune disorders and was not ordered because of his autism diagnosis.

2.2. Subject 1. He is a 13-year-old autistic male with a well-documented history of significant atopic illnesses. He was originally diagnosed with autism by a child neurologist at age of 21 months following a significant regression in child development noted after a viral-like illness with associated high fevers (up to 45.5°C) at age of 15 months. Chronic urticaria, eczema, allergic rhinitis, and asthma have been treated with a variety of antihistamines, montelukast, and topical and systemic steroids since age of 2 years with only temporary beneficial effects. Serum food allergy testing revealed IgE-mediated responses to the following: corn, peanut, soybean, wheat, milk, rice, egg, and numerous other dietary proteins. Both skin prick testing and serum IgE inhalant allergy testing revealed similar patterns of significant reactions to most antigens tested including dust mites and nearly all grasses, trees, weeds, molds, and both domestic and farm animals. Both traditional desensitization with injectable antigens and sublingual immunotherapies have failed to reduce allergic stigmata and had no noticeable effects on the course of the child’s autism. Total serum IgE testing on numerous occasions has been significantly elevated and at the time PEA was introduced; it was 572 IU/mL (nl ≤ 200). Despite the high total levels of IgE and multiple IgE reactants, blood eosinophils have remained in the normal range throughout. Serum vitamin D OH25 levels remain deficient despite extensive efforts at oral supplementation, inferring a defect in absorption of fat-soluble nutrients. Serum vitamin D OH25 levels were at 21 ng/mL at the time PEA was started.

Cellular immune markers reflected specific abnormalities with deficient mature CD57+ natural killer (CD57+ NK) cell counts 32 cells/μL (nl = 60–300 cells/μL), with a total white count of 6.3 × 109/μL. However, the percentage of lymphocytes was high at 58%, while neutrophils were underrepresented at 33%, and absolute lymphocytes were also elevated at 3.5 × 109 cells/μL. All other obtained typical cell indices were in the normal range.

The Childhood Autism Rating Scale, Second Edition (CARS-2) [21], was administered as part of a routine evaluation of the child’s severity prior to a change that is his academic placement. Prior to PEA, CARS-2 scoring placed the child at 43.5 total (82nd percentile) with a verbal communication subscale of 3 out 4. A speech assessment was performed including a mean length of utterance (MLU) measurement. MLU is based on the linguistic concept of morphemes as the smallest component of speech [6]. Speech therapists use MLU as a routine assessment and, in this case, MLU was obtained prior to a change in school placement. MLU was observed to be 3.0 prior to PEA and this corresponded to an age equivalent of approximately 34–35 months (just under a 3-year-old level of speech).
2.3. Post-PEA Supplementation (Subject 1). After the practitioner reviewed the medical literature and the therapeutic rationale with his parents, they treated the child with Normast 600 mg tablets (Epitech Group Srl, Milano, Italy). Normast is available without prescription in Italy and Spain and is classified as “Food for Special Medical Purposes” by the Health Authorities of European Union member states according to standards set forth under European Commission Directive 1999/21/EC.

Initially, 1/2 tablet (300 mg) was given orally with water twice daily on an empty stomach to this boy (a child capable of swallowing tablets). After a week with no observable negative effects, the dose was increased to 1 (600 mg) tablet twice daily. Subject 1’s parents returned for assessment after one month of oral supplementation with PEA. They reported “remarkable” changes in his behavior and his expressive language. Specifically, they noted he was spontaneously joining a conversation and commenting on various things happening in the home. The parents also reported the schoolteacher and speech therapist inquired about what had changed and noted similar positive changes at school. Equally noteworthy was the significant reduction in tantrums, outburst, self-talking, and stereotypes. Further, nose-picking, asthmatic cough, allergy stigmata (dark circles under eyes and nasal itching), nasal edema, and both skin eczema and urticaria diminished clinically after a month of PEA administration. No adverse effects were noted.

Following these comments, we readministered the CARS-2 test and noted a total score of 32 (representing the 28th percentile) whereas the starting score was 43.5 (net change 11.5 points and 54 percentiles less). The subscale for expressive language changed to 2 out 4 and represented significant and noticeable changes. Due to these observations, we suggested reducing the dose of PEA and increasing it to 1 (600 mg) tablet twice daily on an emptystomach to this boy (a child capable of swallowing tablets). After a week with no observable negative effects, the dose was increased to 1 (600 mg) tablet twice daily. Subject 1’s parents returned for assessment after one month of oral supplementation with PEA. They reported “remarkable” changes in his behavior and his expressive language. Specifically, they noted he was spontaneously joining a conversation and commenting on various things happening in the home. The parents also reported the schoolteacher and speech therapist inquired about what had changed and noted similar positive changes at school. Equally noteworthy was the significant reduction in tantrums, outburst, self-talking, and stereotypes. Further, nose-picking, asthmatic cough, allergy stigmata (dark circles under eyes and nasal itching), nasal edema, and both skin eczema and urticaria diminished clinically after a month of PEA administration. No adverse effects were noted.

Following these comments, we readministered the CARS-2 test and noted a total score of 32 (representing the 28th percentile) whereas the starting score was 43.5 (net change 11.5 points and 54 percentiles less). The subscale for expressive language changed to 2 out 4 and represented significant and noticeable changes. Due to these observations, we suggested repeating the MLU assessment for further definition of the degree of language changes. Reassessment of MLU also changed significantly to 5.4 (approximately 58 month’s age-equivalency). This represented a 2-year age gain in expressive language in only one month of supplementation with PEA.

Total serum IgE testing after PEA was introduced remained essentially unchanged at 567 IU/mL (nl < 200). Serum vitamin D-OH25 levels rose to 46 ng/mL after PEA was started (possibly due to better absorption). CD57⁺ natural killer (CD57⁺ NK) cell counts increased after PEA to 52 cells/μL (nl = 60–300 cells/μL), with a total white count remaining similar at 5.6 × 10^9/μL. Noticeably the percentage of lymphocytes reduced to 44%, and the other cell indices were also in the normal range.

2.4. Subject 2. He is an Italian 15-year-old male, whom developed normally for the first 2 years, but near 3 years of age, the child developed a progressive loss of previously acquired language, eye contact, and social interaction. Ultimately, this was diagnosed with autism spectrum disorder. At 6 years of age, he experienced his first partial complex epileptic episode, described as the sudden onset of unconsciousness with resultant loss of motor control. The child fell and struck his head although no apparent concussion occurred. He was eventually treated with sodium valproate. The last episode of epilepsy was in March 2006. No recurrent seizures have been reported. After this event the family initiated dietary restriction by eliminating gluten, casein, yeast, sugar, and soy, and they also began supplementation of a multivitamin and additional folic acid. He started diet restriction and vitamins since 2007 at age of 8 years and never stopped. Following these changes the child’s overall tone and energy improved and sensory integration also became more appropriate.

The Autism Treatment Evaluation Checklist (ATEC) [22] test was administered as part of a routine evaluation of the child’s severity prior to a change in his academic placement. The ATEC has been shown to correlate with the CARS test in domains of sensory/cognitive awareness, speech/language, and sociability [23]. Prior to PEA supplementation the ATEC total scoring was in the mild to moderate range, specifically 25 (a lower number is better): speech 6 of 28 points, sociability 3 of 40 points, sensory/cognitive 1 point (normal), and overall health/behaviors 15 of 75 points. Total serum IgE testing was in the normal range at 127 IU/mL (nl < 200). Serum vitamin D-OH25 levels were normal at 45.10 ng/mL at the time PEA was started. We conducted the observations until three months after treatment. The boy never stopped diet restrictions and multivitamin supplementation since 2007.

2.5. PEA Supplementation. After practitioner NA reviewed the medical literature and the therapeutic rationale with his parents, they treated the child with Normast 600 mg tablets (Epitech Group Srl, Milano, Italy). After three months of supplementation, ATEC total score reduced by half (12 (after) versus 25 (before) points). The individual scores in the separate domains were speech 4 points, sociability 1 point, sensory/cognitive 0 points, and overall health/behaviors 7 points (Figure 1, Table 1). Also shown for comparison in (Figure 1, Table 1) are the child’s improvements in aggression and cognitive and behavioral skills. Language showed mild improvements. In addition, in this case, total serum IgE reduced by half (66.0 post compared to 127 IU/mL pre-PEA). No adverse effects were noted (i.e., nausea, fever, hyperactivity, sleep disorders, skin rash, allergic reactions, and gastrointestinal problems).

3. Discussion

Other anti-inflammatory agents beyond those already discussed have been studied in autism. Spironolactone is a potassium sparing diuretic and an anti-inflammatory which down-regulates nuclear factor-κB [24]. Bradstreet et al. reported the first case of significant reduction in autism severity and improved expressive language after a short course of spironolactone [25]. Bradstreet et al. also reported favorable responses in children with autism treated with transplantation of fetal stem cells [26]. With similar immunological properties, human cord blood mononuclear cells and umbilical cord-derived mesenchymal stem cells were transplanted into children with autism and again positive benefits were noted [27]. Celecoxib, a cyclooxygenase-2 inhibitor anti-inflammatory, plus risperidone, demonstrated significantly superior effects in irritability than risperidone alone [28].

We have previously shown that circulating monocytes in ASDs express abnormal EC2 activity when compared
Table 1: Score distributions on a scale of Autism Treatment Evaluation Checklist (ATEC) in IF autistic patient before (baseline) PEA supplementation and three months later.

|                  | Speech (normal range 0–28) | Sociability (normal range 0–40) | Sensory/cognitive (normal range 0–36) | Behaviors (normal range 0–75) | Total score (normal range 0–180) |
|------------------|-----------------------------|----------------------------------|----------------------------------------|--------------------------------|----------------------------------|
| Before PEA supplementation | 6                           | 3                                | 1                                      | 15                             | 25                               |
| After PEA supplementation  | 4                           | 1                                | 0                                      | 7                              | 12                               |

Figure 1: Dynamics on a scale of Autism Treatment Evaluation Checklist (ATEC) in Subject 2 autistic patient before (baseline) PEA supplementation and three months later. The purpose of the ATEC is to measure change in an individual due to various interventions, that is, the difference between the initial (baseline) ATEC scores and later ATEC scores [6].

Endocannabinoids (EC), including PEA, play an important role in stress and trauma. Individuals with posttraumatic stress disorder (PTSD) had significant alterations of their EC levels, and PEA blood levels were significantly higher in the PTSD population than in controls [32]. In the future, EC- and PEA-commercial blood testing may be a valuable biomarker and a useful tool in predicting and evaluating treatment response to oral supplementation.

Endocannabinoids (EC), including PEA, play an important role in stress and trauma. Individuals with posttraumatic stress disorder (PTSD) had significant alterations of their EC levels, and PEA blood levels were significantly higher in the PTSD population than in controls [32]. In the future, EC- and PEA-commercial blood testing may be a valuable biomarker and a useful tool in predicting and evaluating treatment response to oral supplementation.

In the United States, recent prevalence data indicate greater than 2% of boys may have ASDs [35, 36]. Given the urgent need for safe and effective strategies for treating ASDs, we propose appropriate double-blind controlled clinical trials to explore further the potential for PEA efficacy and its safety.

4. Conclusion

In this original report of two cases, we show PEA-mediated effects may be beneficial for treating core symptoms of autism. PEA is a well-studied, apparently safe even in the pediatric population, anti-inflammatory capable of regulating mast cells and modulating immune chemistry. It is manufactured in the EU and prepared under strict standards according to Good Manufacturing Practice (GMP). PEA also appears to be a atypical endocannabinoid, with additional anti-inflammatory effects mediated via the PPAR pathway. In the United States, recent prevalence data indicate greater than 2% of boys may have ASDs [35, 36]. Given the urgent need for safe and effective strategies for treating ASDs, we propose appropriate double-blind controlled clinical trials to explore further the potential for PEA efficacy and its safety.

Abbreviations

AD: Atopic dermatitis
ADHD: Attention-deficit/hyperactivity disorder
ASDs: Autism spectrum disorders
ATEC: Autism treatment evaluation checklist
BP: Bipolar disorder
Acknowledgments

This study is dedicated to the memory of Professor James Jeffrey Bradstreet who passed away on June 19, 2015. His contribution to this work is fundamental. We thank the two families who volunteered their children’s cases. The authors gratefully thank Ms. Giovanna Gallone and the non-profit organization “Cancellautismo,” Italy, for the useful assistance.

References

[1] A. Masi, D. S. Quintana, N. Glozier, A. R. Lloyd, I. B. Hickie, and A. J. Guastella, “Cytokine aberrations in autism spectrum disorder: a systematic review and meta-analysis,” *Molecular Psychiatry*, vol. 20, pp. 440–446, 2015.

[2] O. Zerbo, C. Yoshida, J. K. Grether et al., “Neonatal cytokines and chemokines and risk of Autism Spectrum Disorder: the Early Markers for Autism (EMA) study: a case-control study,” *Journal of Neuroinflammation*, vol. 11, article 113, 2014.

[3] R. H. Mitchell and B. I. Goldstein, “Inflammation in children and adolescents with neuropsychiatric disorders: a systematic review,” *Journal of the American Academy of Child & Adolescent Psychiatry*, vol. 53, no. 3, pp. 274–296, 2014.

[4] S. Gupta, “Immunological treatments for autism,” *Journal of Autism and Developmental Disorders*, vol. 30, no. 5, pp. 475–479, 2000.

[5] M. Boris, C. C. Kaiser, A. Goldblatt et al., “Effect of pioglitazone treatment on behavioral symptoms in autistic children,” *Journal of Neuroinflammation*, vol. 4, article 3, 2007.

[6] J. F. Miller and R. S. Chapman, “The relation between age and mean length of utterance in morphemes,” *Journal of Speech, Language, and Hearing Research*, vol. 24, no. 2, pp. 154–161, 1981.

[7] J. D. Lewis, A. Ferrara, T. Peng et al., “Risk of bladder cancer among diabetic patients treated with pioglitazone: interim report of a longitudinal cohort study,” *Diabetes Care*, vol. 34, no. 4, pp. 916–922, 2011.

[8] J. M. Keppel Hesselink, “Evolution in pharmacologic thinking around the natural analogic palmitoylethanolamidade: from nonspecific resistance to PPAR-agonist and effective nutraceuti-
cal,” *Journal of Pain Research*, vol. 6, pp. 625–634, 2013.

[9] L. Aloe, A. Leon, and R. Levi-Montalcini, “A proposed autacoid mechanism controlling mastocyte behaviour,” *Agents and Actions*, vol. 39, no. 1, pp. C145–C147, 1993.

[10] H. Jyonouchi, L. Geng, A. Ruby, and B. Zimmerman-Bier, “Dysregulated innate immune responses in young children with autism spectrum disorders: their relationship to gastrointestinal symptoms and dietary intervention,” *Neuropsychobiology*, vol. 51, no. 2, pp. 77–85, 2005.

[11] S. J. Walker, J. Fortunato, L. G. Gonzalez, and A. Krigsman, “Identification of unique gene expression profile in children with regressive autism spectrum disorder (ASD) and ileocolitis,” *PLoS ONE*, vol. 8, no. 3, Article ID e58058, 2013.

[12] L. de Magistris, V. Familiari, A. Pascotto et al., “Alterations of the intestinal barrier in patients with autism spectrum disorders and in their first-degree relatives,” *Journal of Pediatric Gastroenterology and Nutrition*, vol. 51, no. 4, pp. 418–424, 2010.

[13] F. Borrelli, B. Romano, S. Petrosino et al., “Palmitoylethanolam-
amide, a naturally occurring lipid, is an orally effective intestinal anti-inflammatory agent,” *British Journal of Pharmacology*, vol. 172, no. 1, pp. 142–158, 2014.

[14] G. M. Rasò, R. Russo, A. Calignano, and R. Meli, “Palmitoyle-
ethanolamide in CNS health and disease,” *Pharmacological Research*, vol. 86, pp. 32–41, 2014.

[15] K. Mašek, F. Perlík, J. Klíma, and R. Kählch, “Prophylactic efficac-
y of N-2-hydroxyethyl palmitamide (Impulsin) in acute respiratory tract infections,” *European Journal of Clinical Pharc-
amology*, vol. 7, no. 6, pp. 415–419, 1974.

[16] R. Kählch, J. Klíma, F. Čihá et al., “Studies on prophylactic efficacy of N-2-hydroxyethyl palmitamide (Impulsin) in acute respiratory infections. Serologically controlled field trials,” *Journal of Hygiene Epidemiology Microbiology and Immunology*, vol. 23, no. 1, pp. 11–24, 1979.

[17] V. Plesník, M. Havráňová, J. Jancová, J. Januska, and O. Macková, “Impulsin in the prevention of acute respiratory diseases in school children,” *Česko-Slovenska Pediatric*, vol. 32, no. 6, pp. 365–369, 1977.

[18] D. Wiedermannová, D. Wiedermann, and J. Lokaj, “Prophyl-
actic administration of impulsin to clinically healthy children. Effect on the serum proteins and metabolic activity of granulocytes,” *Casopis Lekaru Českych*, vol. 117, no. 33, pp. 1030–1034, 1978.
[19] T. C. Theoharides, A. Angelidou, K.-D. Alysandratos et al., “Mast cell activation and autism,” *Biochimica et Biophysica Acta: Molecular Basis of Disease*, vol. 1822, no. 1, pp. 34–41, 2012.

[20] S. D. Skaper, L. Facci, and P. Giusti, “Mast cells, glia and neuroinflammation: partners in crime?” *Immunology*, vol. 141, no. 3, pp. 314–327, 2014.

[21] E. Schopler and M. E. Van Bourgondien, *Childhood Autism Rating Scale*, CARS-2, 2nd edition, 2010, http://www.wpspublish.com/.

[22] B. Rimland and M. Edelson, *Autism Treatment Evaluation Checklist*, Autism Research Institute, San Diego, Calif, USA, 1999, http://autismeval.com/ari-atec/report1.html.

[23] D. A. Geier, J. K. Kern, and M. R. Geier, “A comparison of the autism treatment evaluation checklist (ATEC) and the childhood autism rating scale (CARS) for the quantitative evaluation of autism,” *Journal of Mental Health Research in Intellectual Disabilities*, vol. 6, no. 4, pp. 255–267, 2013.

[24] Y. Kato, H. Kamiya, N. Koide et al., “Spironolactone inhibits production of proinflammatory mediators in response to lipopolysaccharide via inactivation of nuclear factor-κB,” *Immunopharmacology and Immunotoxicology*, vol. 36, no. 3, pp. 237–241, 2014.

[25] J. J. Bradstreet, S. Smith, D. Granpeesheh, J. M. El-Dahr, and D. Rossignol, “Spironolactone might be a desirable immunologic and hormonal intervention in autism spectrum disorders,” *Medical Hypotheses*, vol. 68, no. 5, pp. 979–987, 2007.

[26] J. J. Bradstreet, N. Sych, N. Antonucci et al., “Efficacy of fetal stem cell transplantation in autism spectrum disorders: an open-labeled pilot study,” *Cell Transplantation*, vol. 23, supplement 1, pp. 105–112, 2014.

[27] J. Baio, Developmental Disabilities Monitoring Network Surveillance Year 2010 Principal Investigators, Centers for Disease Control and Prevention (CDC), Atlanta, Ga, USA, 2014.

[28] Centers for Disease Control and Prevention, “Prevalence of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 sites, United States, 2010,” *Morbidity and Mortality Weekly Report: Surveillance Summaries*, vol. 63, no. 2, pp. 1–21, 2014.