

Spironolactone Might be an Ideal Immunological and Hormonal Intervention in Autism Spectrum Disorders

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Abstract

Multiple studies demonstrate that autism is characterized by immune system dysregulation with evidence of neuroglial activation and gastrointestinal inflammation. This neuroglial process has further been characterized as neuroinflammation. In addition, a subset of autistic children exhibit higher than average levels of androgens. Spironolactone is an aldosterone antagonist and potassium-sparing diuretic with a desirable safety profile. Its potent anti-inflammatory and immune modifying properties might make it an ideal medical intervention in autism spectrum disorders. Furthermore it possesses significant anti-androgen properties which might further enhance its appeal in autism, particularly in a definable subset of hyperandrogenic autistic children. One case report is briefly reviewed demonstrating objective clinical improvements in an autistic child after spironolactone administration. Additional research in controlled trials is now needed to further define the risks and benefits of spironolactone use in children with autism.

Key Words: autism; autistic spectrum disorders; spironolactone; androgen; testosterone; neuroglial; neuroinflammation; inflammation; immune dysregulation

Background

Classical thinking regarding autism defines it as a developmental disorder with abnormalities in language, socialization, and stereotypical unusual behaviors. Autism spectrum disorders (ASD) are increasingly reported as being both common and linked to various triggers. Two recent studies demonstrate that the prevalence of autism is increasing, with indications that as many as 1 to 2% of boys might be affected [1-2]. Both studies confirm observations that approximately 4 times as many males are affected as females. This spectrum of disorders is also widely recognized as having a degree of gene–environment interaction as recently reviewed by the CHARGE study [3]. Furthermore, a genetic link to the Y chromosome axis is lacking, implying a likely hormonal vulnerability to certain environmental triggers. Abnormally high levels of androgens have been an inconsistent finding in autism, however several studies confirm that at least a subset of ASD children can be documented to have either elevated testosterone and/or dehydroepiandrosterone (DHEA) levels [4-6]. In addition, many children with autism have evidence of neuroinflammation and gastrointestinal inflammation.

Hypothesis

Autism is characterized by both increased inflammation and immune dysregulation. In addition, a subset of autistics exhibit higher than average androgen levels. Spironolactone is an aldosterone antagonist and potassium-sparing diuretic that possesses potent anti-inflammatory, immune, and hormone modifying properties which might make it an ideal medical intervention in autism spectrum disorders.

Evidence and Discussion

Immune Dysregulation in Autism

Recognition has been growing over the past 30 years that autism spectrum disorders are often associated with abnormal immune function. In 1976, Stubbs first noted the absence of a normal response to rubella vaccination in a group of children with autism [7]. Then in 1977, Stubbs and Crawford went on to demonstrate suppressed responsiveness to *in vitro* phytohemagglutinin stimulation of lymphocyte cultures [8]. In as early as 1982, Weizman and colleagues demonstrated abnormal cell-mediated immune response to brain tissue in ASD [9]. The understanding of cell-mediated immune dysfunction in ASD was expanded by 1986 to include reduced responsiveness in lymphocyte blastogenesis, decreased numbers of T-lymphocytes, and an altered ratio of helper to suppressor T cells [10]. Warren next demonstrated reduced natural killer cell activity in ASD affected children [11]. As further evidence regarding immune abnormalities mounted in the neuroimmunopsychiatry literature [12-14], Francesetti and colleagues advanced the hypothesis that the immune system may play a role in the pathogenesis of both schizophrenia and infantile autism [15].

By the 1990's, the science of immunology was rapidly expanding, spurred on by the successes of AIDS-related research and large governmental grants. This permitted a more advanced investigation of the neuroimmune aspects of autism. In 1991, Singh et al first reported abnormal cytokine levels which indirectly indicated activation of a subpopulation of T-cells in autism [16]. The autoimmune aspect of autism was furthered by Warren et al when they discovered abnormalities in T cells compatible with an autoimmune disorder [17]. Scifo et al reported a favorable decrease in autism symptoms directly related to the normalization of immune abnormalities following intervention with naltrexone [18]. This treatment resulted in a significant increase in the number of T-helper-inducer cells (CD4+CD8-) and a substantial

reduction in the number of T-cytotoxic-suppressor cells (CD4-CD8+), thereby normalizing the CD4/CD8 ratio. Singh reported increased levels of interferon gamma and interleukin 12 and interpreted these results as a further indication of autoimmunity [19]. This autoimmune theory of autism was again reinforced by the observations of increased levels of urinary neopterin and biopterin, which were compatible with cell immune activation [20]. Adding more weight to this argument were the observations by researchers at Washington University in St. Louis of autoantibodies to the brain endothelium in autistics [21]. Connolly and associates then defined the association of brain directed sera autoimmune markers, including elevations of brain derived neurotrophic factor (BDNF), with autism [22]. Recently, researchers at the University of Cincinnati found a blood cytokine profile consistent with increased activation of both the Th1 and Th2 arms of the adaptive immune response, with Th2 predominance, but without the compensatory increase in the regulatory cytokine IL-10 [23].

Simultaneous to this backdrop of brain-related immune investigations, other researchers were studying the link between dietary proteins and autism. Jyonouchi et al observed that children with autism produced an excess of tumor necrosis factor alpha (TNF-alpha) in response to dietary proteins and bacterial endotoxin–lipopolysaccharide [24, 25]. Of further interest in related research, others detected likely cross-reactivity of the wheat derived protein, gliadin, and Purkinje cell peptides [26]. The researchers noted an eight amino acid shared sequence which would be more than adequate to trigger immune cross-reactivity and hence autoimmunity. This research would still fall short of a direct mechanism of peripheral activation of the immune system triggering neurodevelopmental disruption, but the immune state of autism was becoming clearer.

As the immune disruptions in autism were being defined, another pathway of immune research looked at the observations of a distinct inflammatory bowel disease [27] and other gastrointestinal disorders, including abnormal carbohydrate digestion and reflux esophagitis [28]. While this remains an emotionally charged debate in medicine secondary to issues surrounding purported measles, mumps, and rubella vaccine reactions [29], the observations of a panenteric bowel disease are now well recorded in the medical literature [30]. The gastrointestinal inflammatory features of autism are now finding greater acceptance by diverse investigators [31-33].

More recently, focus has intensified on the immune findings in the autistic brain which have been assisted by greater access to autism brain tissue samples, which help researchers look into the details of the immunological changes within the autistic brain. In what appears to be a landmark study, Vargas et al observed neuroglial activation and evidence of inflammation in both children and adults with autism [34]. Their observations were of unquestioned importance and included marked activation of microglia and astroglia. They also described cytokine profiling and indicated that macrophage chemoattractant protein-1 (MCP-1) and transforming growth factor beta-1, derived from neuroglia, were the most prevalent cytokines in autistic brain tissues. Furthermore, the cerebrospinal fluid (CSF) showed a unique proinflammatory profile of cytokines, including a marked increase in MCP-1 and interferon gamma. Intriguingly, researchers from the same institution were not able to reproduce similar findings in the CSF of a different population of autistic children in a later study [35]. The original study differed from the latter by specifically looking at brain tissue levels of immune activators as well as documenting the histological changes in both microglia and astroglial cells. The original study also histologically documented perivascular neuroglial activation in the autistic brain. This

observation is consistent with either astrocytic defense of the blood-brain barrier or autoimmune activation, and is concerning in light of the previously mentioned endovascular autoimmunity reported by Connolly et al.

A favorable clinical response to immune modification in light of the serological and immune cellular abnormalities found in autism supports the underlying immune-mediated etiology of the disorder and also encourages interest in immunological interventions. Gupta et al observed both immune dysregulation and a favorable response to treatment with human intravenous immunoglobulin (IVIG) [36]. Not all investigators have been able to reproduce this favorable of a response to IVIG, but subpopulations of immune deficient children with autism have been reported to respond well to IVIG in the majority of cases [37]. Several other researchers have reported encouraging clinical improvements by the elimination of certain dietary proteins which are now presumed to be potent inducers of proinflammatory cytokines. A study in 1978 evaluated the effects of a carefully constructed elimination–reintroduction diet on disruptive and hyperactivity behavior in an 8 year-old autistic boy [38]. Results showed that foods such as wheat, corn, tomatoes, sugar, mushrooms, and dairy products were instrumental in producing behavioral disorders in this child. Several years before, Jyonouchi and colleagues noted a marked increase in TNF-alpha in response to milk proteins [24, 25]. In another study, 36 Italian children with autism were evaluated for milk-related antibodies and response to milk elimination as compared to 20 control children [39]. The researchers noted higher antibody levels to milk proteins in the autistic population and a marked improvement in the behavioral symptoms of autistic patients after a two month milk elimination diet. Dr Cade et al, at the University of Florida reported a significant improvement in 81% of children with autism who went on a gluten and casein elimination diet [40]. A Norwegian cohort followed for four years

with a similar diet also showed significant improvements in cognitive abilities and communicative skills [41]. Gupta reviewed the medical literature on various immunological interventions for autism including: transfer factor, which has been shown to confer immunity; pentoxifylline, a phosphodiesterase inhibitor, which is known to have immunomodulatory effects; and IVIG which is concentrated immunoglobulin, primarily IgG, and has been shown to be beneficial in other neuroimmune disorders as well as epileptic disorders [42]. As mentioned previously, Scifo et al found that the therapeutic benefit of low dose naltrexone in children with autism was associated with a reduction in cytotoxic T-cells and the normalization of the CD4/CD8 ratio.

Typically, in refractory immune activation disorders, especially autoimmune disorders, clinicians eventually attempt systemic corticosteroids in an attempt to down-regulate the immune system. Little has been published regarding this intervention for autism; however, in an intriguing case-report, Shenoy et al described a child who rapidly developed autism with the onset of autoimmune lymphoproliferative syndrome (ALPS) [43]. Low-dose steroid treatment resulted in rapid relief of the ALPS and eventual complete remission of the autism as well. Another case report also demonstrated significant improvement following corticosteroids in a child with pervasive development disorder [44].

Evidence of Elevated Androgens in a Subset of Autistics

Data from several sources on the possible role of elevated androgens in aggressive behaviors, and possibly in the pathogenesis of autism, lends support to interventions that reduce androgenic activity as well. In addition to their published works on the subject, Geier and Geier have presented their unpublished clinical findings regarding leuprolide acetate, a synthetic

nonapeptide analog of naturally occurring gonadotropin-releasing hormone, as an intervention for children with concurrent autism and elevated androgens [45]. While the application of the diagnosis of precocious puberty to the Geiers' reported population is as controversial as the intervention itself, the parental and clinical observations are at least intriguing. Of concern is the simultaneous treatment with Succimer (DMSA), a heavy metal chelator, in the children receiving leuprolide acetate. Without delving into the mercury-based research in autism, using a chelator with leuprolide acetate makes attributing the observed benefits to hormone manipulation more challenging. Despite this controversy, others have found leuprolide acetate beneficial in the management of troubling sexual behaviors in autism [46].

The Potential Use of Spironolactone in Autism

Given the large body of experimental and clinical observations regarding immunological and hormonal issues in autism, an ideal interventional agent would simultaneously address both issues. This agent might have the following properties: 1) downregulation of the TNF-alpha response to provoking agents, 2) decrease in MCP-1 and interferon gamma in the brain with resultant decrease in glial activation and inflammation, 3) decrease in inflammation in the gastrointestinal tract, 4) and decreased androgenicity without interference of normal growth. Given these criteria, spironolactone would appear to have nearly ideal properties. Spironolactone is an aldosterone antagonist available only by prescription. It is generally known as a potassium sparing diuretic and has had a lengthy period of observation in both children and adults. Its common and indicated uses are for congestive heart failure, hyperaldosteronism, edema associated with liver failure, nephrotic syndrome, hypertension, and hypokalemia (low serum potassium). It is considered safe for children when used in typical doses (i.e., 1-3 mg/kg/day)

[47], although the official FDA literature states that a safe range has not been established. The oral LD50 of spironolactone is greater than 1,000 mg/kg in mice, rats, and rabbits [48].

Spironolactone was first known to possess anti-inflammatory properties as early as 1961 [49]. However that observation seems to have gone largely unnoticed until the last few years. In a recent study, Japanese researchers looking for a reduction in cardiovascular risk factors related to inflammation found spironolactone to be the most potent anti-inflammatory medication they studied [50]. Specifically, it was found to potently reduce both TNF-alpha and MCP-1 in cultured human monocytes. These effects occurred at levels obtainable during routine oral administration of the medication. In a Danish population of rheumatoid arthritis patients (including juvenile idiopathic arthritis), a modest dose of 1-3 mg/kg/day resulted in a significant reduction of proinflammatory cytokines as well as decreased gene transcription for many regulators of inflammation [51]. Additionally in this study, incubated human whole blood, treated with spironolactone, demonstrated dramatically reduced interferon gamma and substantially reduced TNF-alpha. None of the juvenile arthritis patients had to withdraw from this study and 8 out of 9 children (mean age 12) responded favorably to spironolactone at a dose range of 2-3 mg/kg/day. One female patient had disruption of her menstrual cycle, a known side-effect. Further, in type 2 diabetics, spironolactone administration attenuated elevated MCP-1 levels and alleviated oxidative stress [52].

In an extensive literature analysis (Cochrane Review) of the use of spironolactone for hirsutism and acne in female patients, the reviewers concluded that six months of treatment with spironolactone (100 mg/day) was associated with a statistically significant subjective reduction in abnormal hair growth when compared to placebo [53]. Furthermore, spironolactone at a dose of 100 mg/day was superior to either finasteride or cyproterone acetate (other common anti-

androgens) and had little effects on DHEA, DHEA-S, or testosterone levels in the studies evaluated. This is because the mechanism of action of spironolactone occurs by attachment to dihydrotestosterone (DHT) receptors on cell surfaces, thereby inhibiting the binding of DHT to these receptors [54].

Other authors have termed the recent discovery of the immunological benefits of spironolactone as its renaissance [55]. Our clinical experience with spironolactone and autism leads us to believe it might be successful in this population as well. As an example, a 12 year old boy with well-established autism, immune dysregulation, food allergies, and elevated testosterone levels demonstrated significant reduction in the severity and frequency of several aberrant symptoms within four weeks of spironolactone administration at a daily dose of 2 mg/kg. Specifically, administrations of the Aberrant Behavior Checklist (ABC) [56] before and after the implementation of spironolactone indicated a 79% improvement in irritability, a 83% decrease in lethargy, a 60% reduction of stereotypy, a 72% reduction of hyperactivity, and a 67% decrease in inappropriate speech (see Figure 1). In addition, pre- and post-administrations of the Peabody Picture Vocabulary Test III [57] demonstrated a receptive language gain of 21 months in this same four week period, indicating an increase in vocabulary greater than one standard deviation at either age level. Levels of immunoglobulins and testosterone measured immediately prior to treatment with spironolactone 100 mg daily (approximately 2 mg/kg/day) confirmed immune dysfunction and elevated androgen (see Table 1). These tests were similar to cellular immune parameters and testosterone levels assessed one year prior.

Figure 1: Changes in the ABC subset scores pre- and post-spiro lactone at a dose of 2 mg/kg for 4 weeks

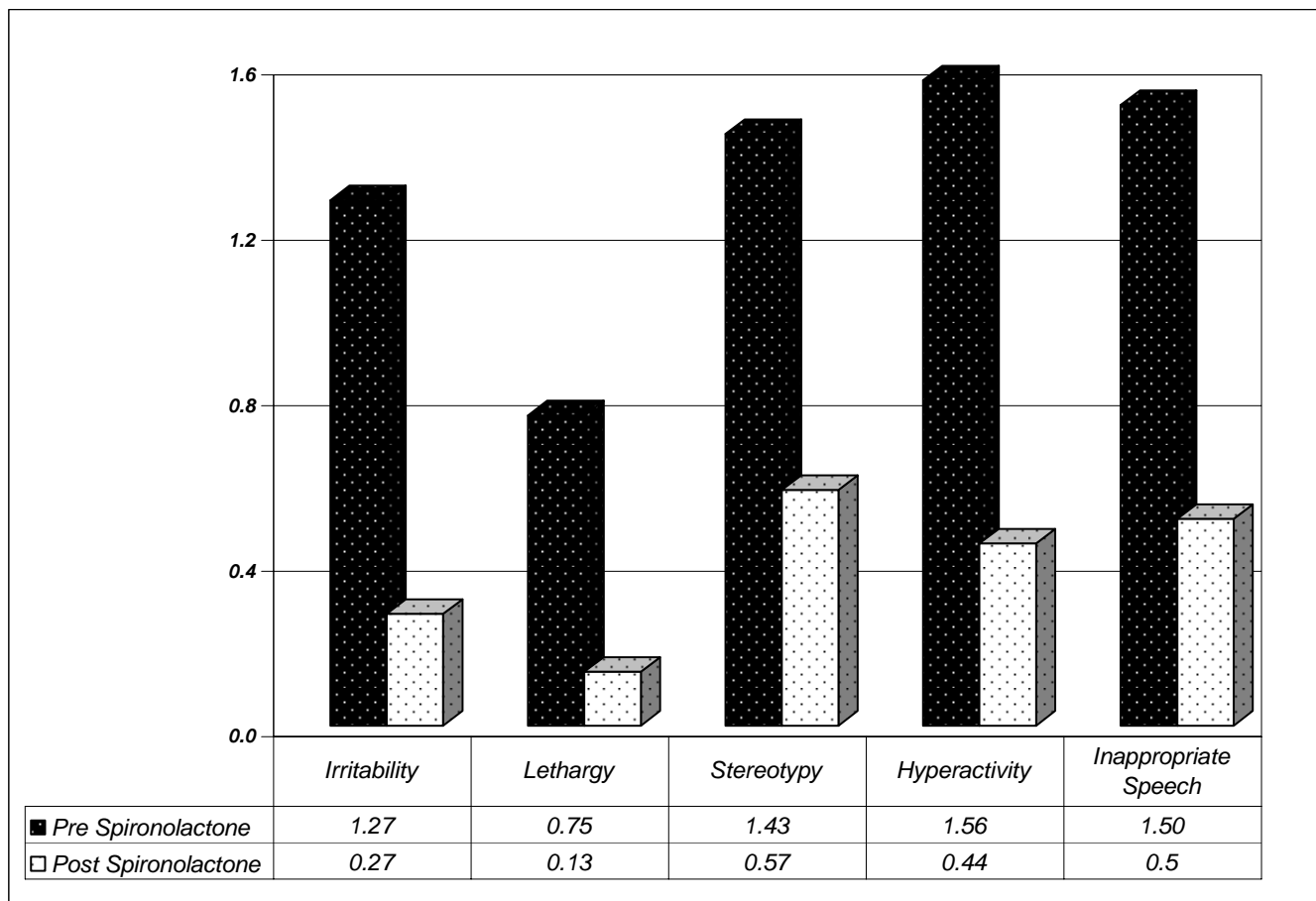


Table 1: Levels of immunoglobulins and testosterone measured immediately prior to treatment with spironolactone

Measure	Patient Level	Reference Range
IgG1	383 mg/dL	389-1372 mg/dL
IgG2	104 mg/dL	125-417 mg/dL
IgG3	24 mg/dL	28-135 mg/dL

IgG4	20 mg/dL	14-162 mg/dL
IgA	85 mg/dL	70-432 mg/dL
IgM	38 mg/dL	52-367 mg/dL
WBC	3.8 thous/mcL	4.5 – 13.5 thous/mcL
Abs CD3	768/CCM	920 – 2200/CCM
Abs CD4	351/CCM	520 – 1440/CCM
Abs CD19+	173/CCM	200 – 820/CCM
Total testosterone	337 ng/dL	< 260 ng/dL
% free testosterone	0.53%	0.53 – 3.33%
Repeat total testosterone	369 ng/dL	< 260 ng/dL
DHEA-S	92 mcg/dL	< 235 mcg/dL

Conclusion

Spirolactone is a low-cost, easily available oral agent with a desirable safety profile, and with nearly ideal immune and inflammatory modification properties. Its secondary benefits as an anti-androgen might further enhance its appeal in autism, particularly in a definable subset of hyperandrogenic children (see Table 2). Additional research in controlled trials is now needed to further define the risks and benefits of spironolactone use in children with autism.

Table 2: Summary of the Use of Spirolactone in Autism

Clinical Finding	Autism Finding	Effect of Spirolactone
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Interferon gamma	↑ [19]	↓ [51]
TNF-alpha	↑ [24, 25]	↓ [50, 51]
MCP-1	↑ [34]	↓ [50, 52]
Inflammation	↑ [27, 34]	↓ [49, 51]
Testosterone	↑ ¹ [4-6]	↓ [53]

¹ Elevated in a subset of autistics

Abbreviations

Autism spectrum disorders — ASD

Dehydroepiandrosterone — DHEA

Brain derived neurotrophic factor — BDNF

Tumor necrosis factor alpha — TNF-alpha

Macrophage chemoattractant protein-1 — MCP-1

Cerebrospinal fluid — CSF

Intravenous immunoglobulin — IVIG

Autoimmune lymphoproliferative syndrome — ALPS

Dihydrotestosterone — DHT

Aberrant Behavior Checklist — ABC

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