Dan Rossignol, MD
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The Use of Hyperbaric Oxygen Therapy in Autistic Children With Special Focus on Inflammation and Oxidative Stress

Summary

Autism is a neurodevelopmental disorder currently affecting as many as 1 out of 166 children in the United States. Numerous studies of autistic individuals have revealed evidence of cerebral hypoperfusion, neuroinflammation and gastrointestinal inflammation, immune dysregulation, oxidative stress, relative mitochondrial dysfunction, neurotransmitter abnormalities, impaired detoxification of toxins, dysbiosis, and impaired production of porphyrins. Many of these findings have been correlated with core autistic symptoms. For example, cerebral hypoperfusion in autistic children has been correlated with repetitive, self-stimulatory and stereotypical behaviors, and impairments in communication, sensory perception, and social interaction. Hyperbaric oxygen therapy (HBOT) might be able to improve one or more of these symptoms in autistic individuals. Specifically, HBOT has been used with clinical success in treating several cerebral hypoperfusion conditions and can compensate for decreased blood flow by increasing the oxygen content of plasma and body tissues. HBOT has been reported to invoke a strong anti-inflammatory response and has been shown to improve immune function. There is evidence that oxidative stress can be reduced with HBOT through the upregulation of antioxidant enzymes. HBOT can also increase the function and production of mitochondria and improve neurotransmitter abnormalities. In addition, HBOT upregulates enzymes that can help with detoxification problems specifically found in autistic children. Dysbiosis is common in autistic children and HBOT can improve this. Impaired production of porphyrins in autistic children might affect the production of heme, and HBOT might help overcome the effects of this problem. Finally, HBOT has been shown to mobilize stem cells from the bone marrow to the systemic circulation. Recent studies in humans have shown that stem cells can enter the brain and form new neurons, astrocytes, and microglia. It is expected that amelioration of these underlying pathophysiological problems through the use of HBOT will lead to improvements in autistic symptoms. Several studies on the use of HBOT in autistic children are currently underway and early results are promising.

HBOT and Autism Overview

At first glance, the use of hyperbaric oxygen therapy (HBOT) in autism appears out of the ordinary. That is what I first thought when I heard about HBOT and autism almost 2 years ago. At the time, no studies existed on the use of HBOT in autistic

individuals. In fact, many people who were proponents for this therapy could not give a theoretical reason why it should even work. We began using HBOT in my 2 children with autism and several others with a great deal of skepticism. After seeing improvements in some of these children, we decided that further study was needed. Just over a year later, we are finishing up our third study on the use of HBOT in autism. We have learned a great deal about how HBOT might be working in autistic individuals and have discovered several main substantial benefits.

To understand how or why HBOT works in autistic children, we need to review some basic, but newly described, fundamental problems found in many autistic individuals. There are now dozens of studies in the medical literature demonstrating cerebral hypoperfusion (decreased blood flow in the brain) in as many as 86% of autistic individuals. Furthermore, this diminished blood flow correlates with core autistic symptoms (see Table 1). It is well-known that when a person has to focus on a task or generate speech (in other words, when the brain has to do work), there is an increase in blood flow to the brain, supplying more blood, oxygen, and glucose (fuel). However, several studies have now demonstrated that not only do some autistic children have diminished blood flow at baseline, they also do not get an increase in blood flow when brain cells have to do more work, such as when the children have to focus on a task or generate a sentence. In fact, sometimes the blood flow goes *down*. The interesting thing about these dozens of studies demonstrating cerebral hypoperfusion is that no one has stopped to ask *why* the diminished blood flow exists in the first place.

Table 1: Selected Areas of Cerebral Hypoperfusion in Autism and Clinical Correlations

Area of Cerebral Hypoperfusion	*Clinical Correlation*
Thalamus	Repetitive, self-stimulatory, and unusual behaviors
Temporal lobes	Desire for sameness and social/communication impairments
Temporal lobes and amygdala	Impairments in processing facial expressions/emotions
Fusiform gyrus	Difficulty recognizing familiar faces
Wernicke's and Brodmann's areas	Decreased language development and auditory processing problems
Temporal and frontal lobes	Decreased IQ

Inflammation in Autism and HBOT

Evidence published out of Johns Hopkins in 2005 demonstrates that, upon autopsy, some autistic children present evidence of inflammation in the brain. Also described was inflammation around blood vessels, which would be consistent with vasculitis, and could cause the vessel wall to become stiff and inflexible. This in turn might decrease the ability of the blood vessel to dilate and lead to diminished blood flow. Furthermore, elevated urinary levels of 8-isoprostane-F2α have recently been described in some autistic individuals. In some studies, this isoprostane elevation has been shown to cause *in vivo* vasoconstriction (decreased diameter of the blood vessel) and increase the aggregation (stickiness) of platelets. A more recent study on autistic individuals also demonstrated increased urinary levels of isoprostane F2α-VI (a marker

of lipid peroxidation, or oxidative stress), 2,3-dinor-thromboxane B2 (which reflects platelet stickiness), and 6-keto-prostaglandin F1α (a marker of endothelium activation, or stickiness of the lining of the blood vessel). These elevated markers indicate that some autistic children have increased platelet aggregation, endothelium activation, and vasoconstriction. Therefore, the inflammation surrounding blood vessels, and the increase in the inflammatory substances leading to vasoconstriction, and increased stickiness of platelets and vessels walls might be causing the diminished cerebral blood flow found in so many autistic individuals.

Inflammation generally is associated with edema (increased swelling), can increase the space between cells, and might increase the amount of fluid present inside cells. Two fMRI (functional Magnetic Resonance Imaging) studies published in 2006 demonstrated that autistic individuals had more fluid inside brain cells when compared to neurotypical children. Furthermore, functional connectivity (the ability of one brain cell to communicate to another) has been shown to be diminished in autistic children versus neurotypical children. It is entirely possible that inflammation present in the brain is leading to diminished blood flow, impaired functional connectivity, and increased fluid inside brain cells as described in these studies.

There have been several other studies in the literature confirming the presence of inflammation in the brain of autistic individuals. Marked activation of microglia and astroglia with elevations in IL-6 and macrophage chemoattractant protein-1 (MCP-1) were found in autistic brain samples upon autopsy, along with increased proinflammatory cytokines in the cerebral spinal fluid (CSF) of living autistic children. Activated microglia have been shown to release inflammatory mediators such as IL-1 and TNF-α, and have been implicated as the primary cell type that controls inflammation-mediated neuronal injury. A cell-mediated immune response to brain tissue in autistic individuals has also been described. In addition, some autistic children have increased glial fibrillary acidic protein (GFAP) in brain samples and the CSF, which is also indicative of inflammation and reactive injury. Autoantibodies to neuron-axon filament protein and GFAP were also increased in the plasma of autistic individuals compared to control individuals. Autistic children make more serum autoantibodies to the brain, including IgG and IgM autoantibodies to brain epithelial cells and nuclei when compared to typical children. Elevated serum autoantibodies to many neuron-specific antigens and cross-reactive peptides have been found in autistic children, including antibodies directed against cerebellar Purkinje cells, and other neural proteins such as myelin basic protein. Furthermore, 49% of autistic children in one study created serum antibodies against the caudate nucleus, and 18% produced serum antibodies to the cerebral cortex. Another recent study demonstrated that autistic children, when compared to control children, developed serum autoantibodies to brain derived neurotrophic factor (BDNF) and had higher levels of serum BDNF. This is important because an elevation of BDNF predicts abnormalities in intellect and social development. Finally, maternal neuronal antibodies might play a role in the development of autism in some children.

Also described in dozens of articles is the presence of inflammation in the intestines of autistic children. This has been termed autistic enterocolitis or chronic ileocolonic lymphoid nodular hyperplasia (LNH). This is characterized by mucosal inflammation of the colon, stomach, and small intestine. As many as 90% of autistic children with gastrointestinal symptoms have evidence of ileal LNH, with 68% having

moderate to severe ileal LNH. In one study, the gastrointestinal mucosa was shown to have increased lymphocytic infiltration and density, crypt cell proliferation, and epithelial IgG deposits mimicking an autoimmune lesion. Another study demonstrated that the gastrointestinal mucosa in autistic individuals had evidence of increased lymphocytes and proinflammatory cytokines including TNF-α and Interferon-γ, and less of the anti-inflammatory cytokine IL-10, which is counter-regulatory. Some autistic children also had evidence of an eosinophilic infiltrate of the gastrointestinal mucosa. Autistic children typically make significantly more serum antibodies against gliadin and casein peptides resulting in autoimmune reactions. More than 25% of autistic individuals make serum IgG, IgM, and IgA antibodies against gliadin, which can cross-react with cerebellar peptides. Furthermore, when compared to typical children, autistic children produce more pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6. Finally, one study has shown that the genetic loci for autism have a propensity to cluster with recognized loci for inflammatory diseases.

Interestingly, autistic children on a gluten and/or casein free diet produced less TNF-α in the colonic mucosa, and had less evidence of eosinophilic infiltration of the mucosa. In addition, review of the literature demonstrates that the use of anti-inflammatory treatments might improve autistic symptomatology. In fact, treatment with corticosteroids of one child who developed an autoimmune lymphoproliferative syndrome and subsequent autism led to objective improvements in speech and developmental milestones. In another child with PDD, whose behavior and language regressed at 22 months of age, treatment with corticosteroids ameliorated abnormal behaviors such as hyperactivity, tantrums, impaired social interaction, echolalia, and stereotypies.

Some autistic children also have evidence of increased immune activation whereby the immune system makes increased amounts of antibodies to body proteins (including brain proteins) that is pathological. Thus, in some autistic individuals, autism might be an autoimmune disease, akin to lupus or rheumatoid arthritis. This is generally reflected by an elevated urinary neopterin which is a marker of increased inflammation and immune activation, and has been found to be elevated in 2 studies of autistic children when compared to neurotypical children. Urinary neopterin levels are very helpful as a screening test for inflammation and immune system activation is autistic individuals.

Treatment of this brain inflammation might help improve the cerebral hypoperfusion found in so many autistic children. In fact, many inflammatory conditions such as lupus, Kawasaki disease, Behçet's disease, encephalitis, and Sjögren's syndrome are characterized by cerebral hypoperfusion, and treatment with anti-inflammatory medication can restore normal cerebral blood flow in some of these conditions. HBOT can overcome the effects of cerebral hypoperfusion (see Table 2) by providing more oxygen to the brain, and by causing angiogenesis of new blood vessels over time by increasing VEGF levels. HBOT might be especially helpful because it provokes a strong anti-inflammatory response as will be discussed in detail shortly. Inflammation is often accompanied by PMN (polymorphonuclear) cell infiltration which can decrease microvascular blood flow; however, HBOT has been shown to decrease the infiltration of PMN's after an ischemic injury to the brain. In addition, HBOT inhibits neutrophil attachment to blood vessel walls, reduces leukocyte adherence, and increases the distance that oxygen can travel in the interstitial space. HBOT has also been used in

cases of vasculitis with good results, and with success in disorders characterized by cerebral hypoperfusion including fetal alcohol syndrome, cerebral palsy, chronic brain injury, closed head injury, and stroke.

Table 2: Proposed Mechanisms of Inflammatory-induced Cerebral Hypoperfusion Found in Autism and HBOT Effects

Autism Inflammatory Finding	*Mechanism of Hypoperfusion*	*HBOT Effect*
↑ 8-isoprostane-F2α and isoprostane $F_{2\alpha}$-VI	Vasoconstriction causes decreased blood flow which leads to decreased delivery of oxygen	Increases the amount of oxygen in plasma and thus increases delivery of oxygen to cells
↑ 2,3-dinor-thromboxane B_2	Increased aggregation of platelets	No effect on platelet aggregation
↑ 6-keto-prostaglandin $F_{1\alpha}$	Endothelial activation	Decreases aggregation of PMN's to endothelium
Cerebral infiltration of perivascular macrophages and microglia	Vasculitis-like condition	Decreases PMN infiltration in injured areas
Cerebral infiltration of perivascular macrophages and microglia	Increased oxygen usage by inflammatory cells and reduced oxygen extraction by normal cells	Increases oxygen in plasma and thus increases delivery of oxygen to cells

HBOT has potent anti-inflammatory tissue effects as revealed by several recent animal studies, with equivalence to diclofenac 20 mg/kg noted in one study. HBOT has been shown to attenuate the production of proinflammatory cytokines including TNF-α, IL-1, IL-1β, and IL-6, and increase the production of anti-inflammatory IL-10. HBOT has also been shown to reduce neuroinflammation in a rat model after traumatic brain injury. HBOT also reduced both inflammation and pain in an animal model of inflammatory pain, decreased the symptoms of advanced arthritis in rats, and attenuated the inflammatory response in the peritoneal cavity caused by injected meconium. HBOT has been used in animal studies to improve colitis, and has been used in humans to achieve remission of Crohn's disease and ulcerative colitis not responding to conventional medications, including corticosteroids. Interestingly, in some studies, the decrease in inflammation with HBOT appeared to be caused by the increased pressure, not necessarily by the increased oxygen tension. In one animal study, hyperbaric pressure without additional oxygen was shown to decrease TNF-α levels. In another human study, HBOT at 2 atmosphere (atm) and 100% oxygen, and hyperbaric pressure at 2 atm and 10.5% oxygen (thus supplying 21% oxygen, equal to room air oxygen) both showed anti-inflammatory activity by inhibiting IFN-γ release by about the same degree, whereas 100% oxygen at room air pressure (1 atm) actually increased IFN-γ release.

The anti-inflammatory effect of HBOT might also occur by decreasing Prostaglandin E2 production which decreases inflammation because prostaglandins increase inflammation, pain, and edema. In one study, HBOT decreased cyclooxygenase-2 (COX-2) enzyme expression after transient cerebral ischemia. The

COX-2 enzyme is responsible for increased prostaglandin production, leading to increased inflammation. Blockade of the COX-2 enzyme has been shown to decrease inflammation and cytokine levels including IL-6. For these reasons, HBOT might help ameliorate the inflammation found in autism (see Table 3).

Table 3: Effects of HBOT on Inflammatory Markers and Inflammation in Autism

Marker	*Classification*	*Autism Finding*	*HBOT Effect*
TNF-α	Inflammatory	↑	↓ [1]
IL-1β	Inflammatory	↑	↓
IL-6	Inflammatory	↑	↓
IL-10	Anti-inflammatory	↓	↑
IFN-γ	Inflammatory	↑	↓ [2]
Neuroinflammation		↑	↓
Gastrointestinal inflammation		↑	↓

[1] Hyperbaric pressure without additional oxygen decreased TNF-α.
[2] Hyperbaric pressure without additional oxygen also decreased IFN-γ.

Oxidative stress in Autism and HBOT

Autistic children present evidence of increased oxidative stress including lower serum glutathione levels. Some autistic children have increased red blood cell nitric oxide, which is a known free radical and toxic to the brain. Lower serum antioxidant enzyme, antioxidant nutrient, and glutathione levels, as well as higher pro-oxidants have been found in multiple studies of autistic children. Autistic children demonstrate evidence of increased lipid peroxidation, including increased malondialdehyde which is a marker of oxidative stress and lipid peroxidation. Decreased activities of certain antioxidant enzymes have also been described in autistic individuals including superoxide dismutase (SOD), glutathione peroxidase, and catalase. Some autistic children also have decreased activity of paraoxonase, an antioxidant enzyme that prevents lipid oxidation and also inactivates organophosphates in humans. The gene for Heat Shock Protein 70 (HSP-70), which protects against oxidative stress, was downregulated in multiple cases of autism. Antioxidants such as ceruloplasmin and zinc tend to be lower in autistic patients, and the ratio of copper to zinc is abnormal in many autistic children. Furthermore, in one study, treatment with antioxidants was shown to raise the levels of reduced glutathione in the serum of autistic children and appeared to improve symptoms. In another study, the use of antioxidants improved behavior in some autistic children.

Concerns have been previously raised that HBOT might increase oxidative stress through the production of reactive oxygen species. This is a relevant concern because of the increased oxidative stress just described in some autistic children. However, oxidative stress from HBOT appears to be less of a concern at pressures under 2.0 atm, which are often used clinically. With long-term and repeated administration, HBOT below 2.0 atm can actually decrease oxidative stress by reducing lipid peroxidation, and by increasing the activity of antioxidant enzymes including SOD, glutathione peroxidase, catalase, paraoxonase, and heme-oxygenase-1. HBOT has also been shown to increase HSP-70, which protects against oxidative stress. One recent animal study

has demonstrated that HBOT can suppress oxidative stress in brain tissues after a stroke. HBOT also increases zinc, decreases copper, and increases ceruloplasmin levels. Thus, HBOT might help improve the oxidative stress found in some autistic individuals.

It must be noted that when a child is undergoing HBOT, there will be a slight increase in free radical production which could lead to oxidative stress. However, oxidative stress will only occur if there are not enough anti-oxidants present to neutralize the free radicals. Oxidative stress is caused by an imbalance of oxidants and antioxidants and does not occur unless the production of free radicals exceeds the ability of the body to neutralize them through anti-oxidants. Furthermore, HBOT itself causes an upregulation in anti-oxidant enzymes as just described. This elevation in anti-oxidant enzyme levels more than adequately compensates for the small increase in free radical production while undergoing HBOT. In typical people without autism, HBOT pressures under 2.0 atm are rarely associated with oxidative stress. However, we know that autistic children are under much more oxidative stress than typical children. Therefore, there is a chance that oxidative stress could happen with HBOT in autistic children, especially at higher pressures. Because of this, we measured markers of oxidative stress in autistic children up to 1.5 atm and 100% oxygen and found that while plasma glutathione levels dropped slightly, there was no evidence of increased oxidative stress inside the cells, as measured by changes in plasma oxidized glutathione. Furthermore, in animal studies, treatment with antioxidants like Vitamin C, melatonin, glutathione, NAC, and others prevented oxidative stress from HBOT at 4.0 atm and 100% oxygen. Consequently, if a child undergoes HBOT at up to 1.5 atm and 100% oxygen and that child is treated with antioxidants, it is very unlikely that the child will experience oxidative stress due to HBOT.