DOI: 10.53660/CLM-2094-23P35

Current Evidences of Therapeutic Effects of Sulforaphane on Oral and Intestinal Microbiota in Autism Spectrum Disorder – ASD

Evidências Atuais dos Efeitos Terapêuticos do Sulforafano na Microbiota Oral e Intestinal no Transtorno do Espectro Autista – TEA

Received: 2023-09-03 | Accepted: 2023-10-05 | Published: 2023-10-11

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ABSTRACT

Growing evidence suggests that the Sulforaphane promising agent against oxidative stress related to Autism Spectrum Disorder (ASD), can reduce signs and symptoms like impaired communication and socialization, stereotyped behaviour and neurodevelopmental abnormalities. The oral and intestinal microbiota is extremely important, when out of balance, is one of the main causes of various desordens in the human organism. Broccoli is one of the functional foods most investigated by science. It contains Sulforaphane, an isothiocyanate with great antioxidant and anti-inflammatory capacity; with studies focusing on neurodegenerative and neurodevelopmental diseases. The bibliographic survey was carried out on the Pubmed platform, between 2013 and 2023, in informations provided for Ministério da Saúde – Brasil and in nutrition books. This is a systematic review on Sulforaphane supplementation for people with ASD and its implications for controlling the symptoms that most affect this population, opens a new avenue for managing autistic patients through non-pharmacological intervention.

Keywords: Oral Microbiota; Phytochemicals; Autism Spectrum Disorder - ASD; Sulforaphane;

RESUMO

Evidências crescentes sugerem que o Sulforafano é um agente promissor contra o estresse oxidativo relacionado ao Transtorno do Espectro Autista (TEA), que pode atenuar sinais e sintomas como comunicação e socialização prejudicadas, comportamento estereotipado e problemas de neurodesenvolvimento. A microbiota oral e intestinal é extremamente importante, quando desequilibrada, é uma das principais causas de diversas desordens no organismo humano. O brócolis é um dos alimentos funcionais mais investigados pela ciência. Contém Sulforafano, um isotiocianato com grande capacidade antioxidante e anti-inflamatória; com estudos voltados às doenças neurodegenerativas e do neurodesenvolvimento. O levantamento bibliográfico foi realizado na plataforma Pubmed, entre 2013 e 2023, em informações fornecidas pelo Ministério da Saúde – Brasil e em livros de nutrição. Essa é uma revisão sistemática sobre a suplementação do Sulforafano para pessoas com TEA e suas implicações no controle dos sintomas que mais acometem essa população, abre um novo caminho, não farmacológico, para o manejo de pacientes autistas.

Palavras-chave: Microbiota Oral; Fitoquímicos; Transtorno do Espectro Autista – TEA; Sulforafano;

INTRODUCTION

Autism Spectrum Disorder (ASD) is mainly characterized by impaired communication, language, and socialization, repetitive, and stereotyped behavior, affecting mainly male individuals, characterized by neurodevelopmental problems. Still without a cure, it is a major challenge with increasing numbers of cases/diagnoses for medicine and science (Singh *et al*., 2014). Neuromodulators that play a key role in emotional regulation, behavior and social interaction are compromised due to alterations in endocannabinoid metabolism in individuals with ASD (Kelly *et al.*, 2019). Half of them are medicated with psychotropic drugs, despite the lack of evidence on their efficacy (Siafis *et al*., 2022).

The increase in the incidence of ASD in several countries has a significant impact on society in general, their families, and especially the individuals themselves, both because of the difficulty of assertive treatment and the cost, due to the lack of knowledge of the processes and procedures that still involve ASD, even the diagnosis itself. The causes are unknown, but believes that there is a strong genetic and epigenetic influence, timing and environment, which in interaction, modulate the development of each individual (Li *et al*., 2019). Despite the diagnostic complexity, also due to the broad profile of the spectrum itself, autism shows signs and symptoms from the first years of life (James *et al.*, 2004).

With extreme clinical relevance, the oral microbiota, when out of balance, is one of the main causes of oral diseases, which can lead to various other diseases, including systemic ones. Its analysis is the key to the diagnosis, onset and progression of pathologies. DNA sequencing methods and other molecular tools improve the understanding of the human oral microbiota, bring about the concept of the "personal microbiome", and contribute to the effectiveness and individualization of medical treatments (Moon *et al.*, 2016).

Studies seek to understand the formation of individuals and conclude that the induction of the mother's immune system, due to various inflammations, such as obesity, stress and gastrointestinal problems, during pregnancy, brings brain damage to the fetus and contributes to the emergence of neuropsychiatric and neurodevelopmental disorders, such as those related to ASD. There is also a significant amount of association with cases of autism in pregnant women who suffered viral infections in the first trimester of pregnancy, and in a smaller number of mothers who suffered bacterial infections in the second trimester of pregnancy. The activation of the immune system seems to happen early, already in the gestational period and remains after birth, which shows a continuous deregulation of this system, with high amounts of cytokines, chemokines and interleukins; a situation related to aberrant behavior and impaired communication; brain and gastrointestinal inflammation (Patterson, 2011).

DNA methylation, histone code modification and microRNA regulation can promote epigenetic changes during development. Patterns in the genome that can be altered shortly after fertilization, and pre-implantation. Epigenetic mechanisms are extremely dynamic, stable but not static, and directly influence cell differentiation. Their deregulation can even trigger the disordered growth of cells, such as cancer cells, but they can be easily modulated by the environment, including diet. Although there is still much to understand about the human epigenome, such as the influence caused by lifestyle and ageing (Berdasco; Esteller, 2010).

Animal studies show that in addition to genetic influences, the maternal intestinal microbiota can be correlated with consequences for the fetus, as it influences the formation of the nervous, endocrine and immune systems, through the gut-brain axis (Kang *et al*., 2017). Situations and treatments that the mother undergoes during prenatal care are also relevant, such as exposure to certain types of drugs and/or toxins (Dietert *et al*., 2011). It has been proven that the microbiota of children with ASD is significantly altered. Research based on other studies suggests that they contain harmful bacterial genera or species that aggravate ASD symptoms. Mothers of children with ASD also have alterations in their gut microbiome (Li *et al.*, 2019).

Epigenetics regulates behavior and gene expression. In nutritional epigenomics, phytochemicals aim to enhance quality of life by modulating gene expression – activating or deactivating them – in a manner beneficial to the individual, without altering the DNA sequence. The most studied epigenetic mechanisms are DNA methylation, modulation of the histone code and microRNAs. Extremely refined and coordinated mechanisms of the human organism, which are greatly influenced by the environment, our lifestyle, emotions, diet and physical activity, directly influencing the microorganisms that inhabit us and consequently ourselves (Bruno; Castro, 2021).

Research on natural compounds aimed at boosting the host’s immunity holds promise for maintaining overall health and improving clinical signs and biochemical markers, with various beneficial properties for humans and animals, including the maintaining of a well-balanced oral microbiota (Mooney *et al.*, 2021). The constant search for preventive health strategies, which help

medical treatments and bring quality of life, has led researchers to study phytochemicals, which are abundant in foods such as fruit, vegetables and legumes, as their constant consumption helps fight diseases due to their antioxidant and anti-inflammatory actions, acting as protectors of the body (Arnoso *et al.*, 2019).

Phytochemicals are present in plant physiology in great quantity and diversity. They play a crucial role in plant growth and defense against parasites and predators. Their colors, aromas and flavors, along with mechanisms of action extending beyond modulating oxidative stress, have demonstrated antioxidant activity. This is associated with a reducing in the manifestation of chronic diseases, including neurodegenerative, cardiovascular diseases, and diabetes mellitus, with antibacterial and antiviral action, such as organosulfur compounds - isothiocyanates, allylic and allylic sulfur compounds. They reduce the action of advanced glycation agents and free radicals, reducing mutagenic lesions and DNA fragmentation (Bruno; Castro, 2021).

According to the Ministry of Health's Virtual Health Library (2009), broccoli is one of the functional foods most investigated by science. Described by its biochemical compounds originating from indoles and isothiocyanates, its main action is as an inducer of antioxidant enzymes. They also include cauliflower, cabbage, Brussels sprouts, radish, turnip and mustard; the Brassicas or Crusciferae (vegetables rich in glucosinolates, compounds that produce Sulforaphane). These foods provide inherent nutritional value through their chemical composition, reducing signs and symptoms of chronic diseases when consumed regularly in moderation as part of a balanced diet and healthy lifestyle. Its inclusion in the diet can bring various benefits to autistic people.

Sulforaphane, an isothiocyanate presente in cruciferous vegetables like broccoli, is a phytochemical known for its potente antioxidant, anti-inflammatory, and mitochondrial protective properties, with studies focused on neurodegenerative and neurodevelopmental diseases. Rich in sulphur, it can cross the blood-brain barrier, express more than 200 antioxidant genes through the induction of the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling cascade, helping in the process of detoxification and neuroprotection of the CNS, reducing reactive oxygen species (ROS) and superoxides. It increases cell apoptosis, inhibits pro-inflammatory cytokines, protects neurons against Aβ42-mediated cytotoxicity, as well as heme toxicity (Aishworiya *et al.*, 2022). The Nrf2 pathway, hypoxia-inducible factor 1 (HO-1), is the target of studies against oxidative stress, considered one of the main means of preserving endothelial cells (Zhang *et al.*, 2021). Its administration in humans is very well tolerated (Kensler *et al.*, 2012).

ASD is usually associated with many cases of autoimmune diseases, gastrointestinal and consequently oral dysbiosis (James *et al.*, 2004). The use of Sulforaphane as a supplementation strategy for autism was based on the large number of studies found that prove its safety and efficacy. There is evidence that the compound neutralizes various biochemical and molecular abnormalities related to ASD, including problems in glutathione synthesis, mitochondrial dysfunction, impaired oxidative phosphorylation, increased reactive oxygen species, lipid

peroxides and neuroinflammation. Even though they are not symptoms for diagnosing ASD, their improvement usually brings significant benefits for these patients and balances the oral microbiota (Aishworiya *et al*., 2022). Assertive treatments for this public are still limited, which is why it is important to develop such studies.

OBJECTIVE

Systematic review on Sulforaphane supplementation for individuals diagnosed with Autism Spectrum Disorder (ASD) and exploring its potential to mitigate the symptoms that significantly impact this population, including neurological, cognitive, behavioral and gastrointestinal aspects.

METHODOLOGY

The research was conducted using the Digital Library of Theses and Dissertations of the University of São Paulo (USP) and the Pubmed platform. Articles considered for this study were published between 2013 and 2023 and were available as Free Full Text. The primary keywords employed were "Sulforaphane," "Sulforaphane and ASD," "Sulforaphane, Oral Microbiota and ASD," "Isothicyanate and Thyroid", and "Sulforaphane and Glucoraphanin". Additionally, relevant books on Nutrition, Health, Phytochemicals, and Healthy Eating were consulted, focusing on Sulforaphane's role in the detoxification process and its influence on the oral and intestinal microbiota. Data from the Ministry of Health, Brazil, were also included in the research. Studies considered to be of low methodological quality, which did not presente clear and reliable criteria, were excluded.

RESULT AND DISCUSSION

The research results were derived from the analysis of 34 articles, data obtained from the Pubmed platform, Digital Library of Theses and Dissertations of the University of São Paulo (USP), data from Brazilian Ministry of Health, and insights from the book *"Chás & Shots"* (Bruno; Castro, 2021), all of which are elaborated in the bibliographical references at the end of this article. The consensus across these sources highlights the beneficial aspects of employing Sulforaphane as a supplement and its interconnected impact on various bodily systems.

The human brain has the ability to analyze particles from plant and animal foods and generate responses. Contact with the nasal cavity, oral cavity, esophagus, stomach and intestine, in order to be absorbed, promotes chain reactions for better use and/or elimination when it reaches the large intestine, also with benefits, interaction and modulation of the local microbiome. Although there is genetic and epigenetic individuality, synergy with the phytochemical and timing, many studies have proven its effects (Bruno; Castro, 2021). A study carried out on

hyperuricemic rats showed that Sulforaphane reduced uric acid and urate synthesis, facilitated renal excretion and metabolism, and consequently increased the diversity of the intestinal microbiota and renal protection by promoting epigenetic changes through Nrf2 (Wang *et al.*, 2022).

As a strategy for diagnosing ASD, new biomarkers are being used to understand the pathophysiology of ASD by discovering changes in amino acids, and reactive oxidative stress, neurotransmitters, and the functioning of the gut-brain axis. Metabolomics studies are being carried out to investigate possible alterations in the metabolic pathways of ASD patients (Likhitweerawong *et al.*, 2021). Good results have been obtained in the search for a reduction in maternal inflammatory processes in pregnant mice, which could affect the fetus, confirming the importance of the search for modulation of the immune system while it is still being formed (Patterson, 2011).

Aisworiva *et al.* (2022) also demontrated that numerous studies have highlighted the positive effects of using Sulforaphane in patients diagnosed with ASD. Many of these studies were carried out with children, who despite not showing a significant improvement in the Ohio Autism Clinical Impressions Scale, showed a significant improvement in biomarkers, including the redox status of glutathione, mitochondrial respiration, inflammatory markers and heat shock proteins with Sulforaphane versus placebo, a condition related to evolution on the ABC (Aberrant Behavior Checklist) scale. Likhitweerawong *et al.* (2021) reinforces that Sulforaphane is capable of improving immunity through the activation of Nrf2, due to its anti-inflammatory and antioxidant actions, effects already found in animal studies.

Due to the strong association of ASD symptoms with oxidative stress pathways (Chauhan; Chauhan, 2006), glycation and protein oxidation, with a large increase in advanced glycation end products (AGEs) - an action that may be related to lipid peroxidation - the use of phytochemicals as an antioxidant strategy is believed to be of interest, with Sulforaphane as a possible way of reversing disordered metabolic pathways related to ASD, mitochondrial dysfunction, and neuroinflammation. Intake of Sulforaphane has been shown to improve not only ASD behavior, but also metabolic regulation, with urine tests showing an increase in antioxidants such as y-glutamylglutamine, methionine sulfone and sphingomyelin, which had previously been found to be reduced in this population, and its restoration is important for improving ASD symptoms (Likhitweerawong *et al.*, 2021).

Sulforaphane as a promising agent in the prevention of diseases related to oxidative stress. Despite concerns regarding potential thyroid toxicity, researchers question this possibility. They argue that it is often based on short and/or inconsistent studies. Moreover, a substantial body of research overwhelmingly supports the benefits of Sulforaphane without any observed harm. In this study, using a drink enriched with Sulforaphane and glucoraphanin, its precursor, biochemical measures of thyroid function and autoimmune thyroid disease were analyzed in 45 women over

84 days. Serum levels of thyroid-stimulating hormones, free thyroxine and thyroglobulin were not affected by the treatment, and the participants' thyroid autoimmunity remained unchanged. The study thus provides evidence in favor of the safety of its administration (Chartoumpekis *et al.*, 2019).

According to studies on crucifers, their consumption needs to be excessive to cause any dysfunction in thyroid function. One example is an 88-year-old Chinese woman who included around 1.0 to 1.5 kg of raw bok choy (a type of Chinese cabbage) in her diet every day for several months to try to control her diabetes. She was admitted to the intensive care unit with a diagnosis of severe hypothyroidism, with a myxedematous coma, despite having no history of previous thyroid problems. Despite the seriousness of the case, she was medicated and recovered. This was because Brassicas contain glucosinolates, and some of the products of their decomposition are thiocyanates, nitriles and oxazolidines, which have inhibitory effects on the thyroid. It is important to remember that Brassicas, when eaten raw, release the enzyme myrosinase, which enhances the hydrolysis effect of glucosinolates. Cooking this type of vegetable deactivates a large part of the effect of this enzyme. The excess and inappropriate use led to all the health complications in the case reported (Chu *et al.*, 2010).

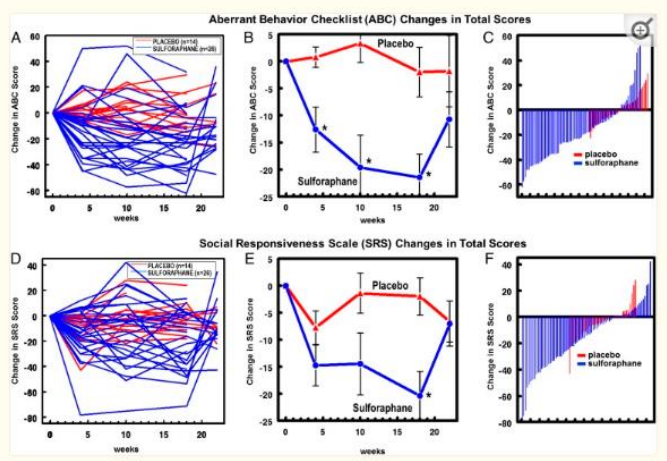
Small molecules such as phytochemicals, including Sulforaphane, have been shown in vitro to have therapeutic potential for the human body, and can bring various benefits by reducing manifestations of genetic disorders, activating the "stress proteome", which regulates harmful events in the body, common in individuals with ASD (Brose *et al.*, 2012). They fight free radicals through antioxidant action by inducing phase II detoxification enzymes such as quinone oxidases, glycuronosyl-S-transferases and glutathione-S-transferases (Holst; Williamson, 2004; Halkier; Gershenzon, 2006; Abdull *et al.*, 2010).

As a result of the hydrolysis of the glucosinolate glicoraphanin (4-methylsulfinibutyl glucosinolate), found in broccoli, the isothiocyanate Sulforaphane (4-methylsulfinyl isothiocyanate) is made available, with potential induction of phase II enzymes, preventing the development of tumors, impeding their cell cycle and bringing about their apoptosis (Thornalley, 2002; Abdull *et al.*, 2010). Also according to Santos, (2019) Sulforaphane has shown great antioxidant potential, even in minimal doses, with an antitumor effect on liver cells, HepG2 and human gastric GAS cells, preventing their development and apoptosis of these cells with higher doses of this phytochemical. The gene expression. of Histone deacetylases (HDAC) was reduced. It is easily absorbed by epithelial cells and rapidly bioavailable. Sulforaphane's anti-tumor and cell-protective capacity has increased interest in its study.

Singh *et al.* (2014), obtained significant results in this study with 26 participants who received treatment with Sulforaphane (daily oral doses of Sulforaphane 50 - 150 µmol, for 18 weeks, with 4 weeks rest, totaling 22 weeks), compared to 14 who received the placebo. Initially, there was not a notable difference; however, by the end of the 18 weeks, a history of improvement emerged,

evidente in significant changes in the total behavioral scores measured by the ABC (Aberrant Behavior Checklist) and SRS (Social Responsiveness Scale). In the Sulforaphane group, there was a 34% reduction in the ABC scores and a 17% reduction in the SRS scores on average, while in the placebo group there was a 3.3% improvement. With a tendency for the ABC and SRS sub-scores to regress to baseline after stopping treatment, as shown in Figures 1 and 2 below.

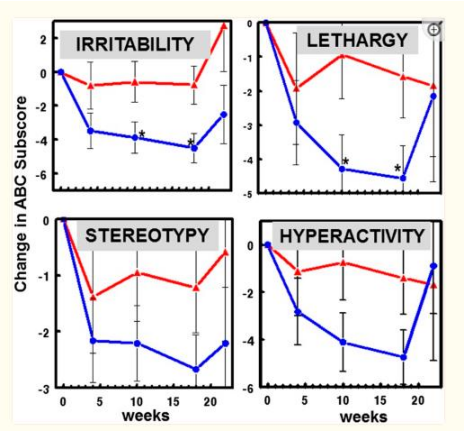
Figure 1 - Representation of the changes in the ABC Test and SRS scores applied to both groups, Placebo (in red) and Sulforaphane (in blue):



Legend: Forty male participants with ASD in the placebo (initially n = 14) or Sulforaphane (initially n = 26) group for 4, 10 and 18 weeks, followed by a terminal 4-week period without treatment (22 weeks). Panels A (ABC) and D (SRS) show all observations. The mean changes in raw and unadjusted total scores (± SEM) at 4, 10, 18 and 22 weeks are shown in B for ABC and E for SRS. Reductions in ABC scores after Sulforaphane treatment were -20.2% (P = 0.035), -31.5% (P = 0.002) and -33.6% (P <0.001) at 4, 10 and 18 weeks, respectively. The changes in SRS were -12.2% (P = 0.29), -12.2% (P = 0.080) and -16.8% (P = 0.017). Panels C (ABC) and F (SRS) show the changes in total scores at all time points for placebo and Sulforaphane participants. The changes were calculated from the initial values individually at time 0 - the average of the two values obtained at screening and enrollment.

Source: Singh K *et al.*, 2014).

Figure 2 - Representation of the results of the changes in sub-scores of the ABC Test and SRS applied to both groups, Placebo (in red) and Sulforaphane (in blue):



Legend: Changes in ABC sub-scores for irritability, lethargy, stereotypy and hyperactivity. After 4, 10 and 18 weeks of treatment with Sulforaphane or placebo, and an untreated recovery period of 4 weeks (22 weeks). Mean raw and unadjusted change values (± SEM) are shown for participants treated with Sulforaphane and placebo. Changes were significant at the 95% confidence level for irritability and lethargy at 10 and 18 weeks.

Source: (Singh K *et al.*, 2014).

Sulforaphane receptors greatly attenuated the signs and symptoms of ASD. 13 participants who received Sulforaphane supplementation demonstrated visible improvements in social interaction, atypical behavior, and verbal communication. Family members and caregivers of the majority of participants who received Sulforaphane 17 compared with 9, out of 26, spontaneously reported the benefits, before they actually knew they were part of the Sulforaphane group, with only 1 of the placebo group revealing that they thought they were receiving the supplementation. Sulforaphane treatment was safe and well tolerated, according to laboratory results. Some adverse effects did occur, but in the minority and in both groups, not directly correlated to the treatment (Singh *et al.*, 2014).

Sulforaphane's therapeutic antioxidant capacity defends cells against toxins, making this herbal medicine an efficient means of detoxification for the body (Egner *et al.*, 2014), as it activates the Keap1-Nrf2 cytoprotective signaling pathway (Kensler *et al.*, 2012). This neuroprotective capacity is highly relevant in cases of ASD (Lyall *et al.*, 2014). According to the demonstrated safety and benefits of Sulforaphane, studies are suggested for the prevention of ASD in prenatal mothers, as well as in children from early childhood (Singh *et al.*, 2014).

Sulforaphane is a cell protector, anti-inflammatory, mitochondrial and synaptic modulator, with neuroprotective mechanisms (Masi *et al.*, 2015; Klomparens; Ding, 2019), and numerous in vitro studies, animal models and clinical studies describe its benefits (Yang *et al.*, 2020). Sulforaphane's ability to activate NRF2 signaling is remarkable, which can modulate the gene

expression of this pathway, with several positive results in humans, such as the reduction of allergic signs and detoxification, one of its most striking effects, as it is an inducer of glutathione and several cellular protective enzymes (Egner *et al.*, 2014).

FINAL CONSIDERATIONS

We hope that further studies will be carried out on Sulforaphane, phytochemicals and strategies aimed at alleviating the signs and symptoms of ASD in this population. We hope that Sulforaphane supplementation will modulate the oral and intestinal microbiota, bringing about beneficial effects on the health of this population. This will be another opportunity to answer questions that have not yet been clarified about the interrelationship between these systems and their possible consequences for the complexity of the signs of this type of disorder.

The scientific scope is broadened as this is a project that involves the correlation between the body's systems, such as the neurological and gastrointestinal, thinking about the needs of this population in ASD, health and well-being for them and their families. It is worth pointing out that the area proposed in the project, oral microbiota applied to human health, is extremely lacking in our country, and this is why this approach is so relevant. We hoped that the development of these strategies will be applied in Brazil, where ASD is a serious and growing issue.

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