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Commentary

Coloring outside the lines: Exploring the dysbiotic impact of azo dyes on gut health and inflammation

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Background

Beyond mere aesthetics, the vibrant hues of fruits, vegetables, and culinary creations evoke sensory experiences that influence perceptions of flavor, freshness, and even nutritional value. This concept highlighted the pivotal role that color can play in the realm of food, serving as a powerful tool to captivate consumers and entice their taste buds.

These facts were discerned by Ancient Egyptians who were pioneers in utilizing natural substances such as saffron, turmeric, and various plant extracts to add vibrant hues to their culinary creations, candies, and wine. The use of these dyes not only enhanced the visual appeal of their dishes but also held symbolic significance in religious rituals and cultural celebrations. In addition, they employed innovative techniques to create artificial colors for food by developing methods to extract pigments from minerals and metals such as malachite for green, ochre for yellow, and cinnabar for red, expanding their palette of culinary shades. However, these artificial colors were often reserved for special occasions and feasts.

Later, the "color revolution" ignited in 1856 with the groundbreaking creation of "mauveine" by British chemist Sir William Henry Perkin through the oxidation of aniline [1]. This vital moment heralded a new era of innovation, birthing thousands of synthetic dyes tailored for industrial applications. Among the most notable developments were artificial colors, which quickly gained prominence as food additives. Their widespread adoption stemmed from several advantages over natural alternatives, including streamlined production, cost-efficiency, enhanced stability, and superior coloring properties [2].

These agents typically fall into two categories: water-soluble dyes and oil-soluble dyes. Water-soluble dyes, often used in beverages and confections, dissolve readily in water-based solutions, while the oil-soluble ones, favored for fats and oils, disperse seamlessly in lipid-rich environments. Manufacture of synthetic food coloring agents stemmed mainly from coal tar, whereas various oil products are used now in their synthesis. The soluble synthetic organic dyes, which add no flavor to the products, include azo dyes, xanthan, chinilin, and antrachinon dyes and their precise chemical synthesis processes are meticulously controlled to ensure purity, consistency, and compliance with regulatory standards [3].

Because of their synthetic chemical origin, heightened inquiry has been directed towards the safety of these additives utilized in food products. Accordingly, the European Parliament and the Council issued regulation (EC) No. 1333/2008 on food additives to address concerns about these agents that

may imperil consumer health, mandating a re-evaluation of the toxicity of food additives assessed before January 20th, 2009, by the European Food Safety Authority (EFSA) (http://eur-lex.europa.eu/legal-content/EN/TXT/?uri¼celex:32008R1333). This systematic reassessment process underscores a commitment to upholding rigorous standards and staying abreast of scientific advancements in understanding the potential risks associated with food additives.

The Double-Edged Sword of Synthetic Colorants

Crosstalk between azo dyes and gut microbiota

Azo dyes, one of the synthetic colorant additives widely utilized in food products, include tartrazine (Yellow 5), Sunset Yellow FCF (Yellow 6), Ponceau 4R, Allura Red AC (Red 40), Quinoline yellow, and Carmoisine, with Red 40, Yellow 5, and Yellow 6 being the most popular ones [4]. Known for their vivid colors, these additives are under scrutiny due to concerns about their potential toxicity. The main worry revolves around the possibility of their conversion into carcinogenic metabolites by intestinal microbiota, which often limits their use [5,6].

Not only these metabolites are mutagenic or carcinogenic substances, but the gut microbiota-mediated degradation of these compounds was also reported to impact the gut and the brain. Indeed, human microbiota, as it develops, operates akin to a secondary brain, impacting neurotransmitter regulation via the vagus nerve in an axis known as microbiota-vagus nerve-brain axis demonstrating bidirectional signaling between the brain and the gut [7]. Pertaining to this, chronic consumption of tartrazine has been found to impair memory and learning in rodents. This effect is attributed to the degradation of the azo dye by gut microbiota, which releases free radicals and consumes ATP. This process ultimately reduces the ability of bacteria to synthesize neurotransmitters and increases oxidative stress [8]. Moreover, a combination of Allura Red AC and amaranth was reported to reduce the neural progenitor cells [9].

Moreover, these dyes can affect children's behavior, because children frequently consume colored products and have underdeveloped blood brain barrier.

Additionally, consumption of these dyes can result in allergies and attention deficit in children, where consumption of Allura Red AC has been reported to stimulate brain histamine levels after altering histamine metabolizing genes [10]. Whether these central changes are linked to Azo dyes-mediated dysbiosis or to the gut microbiome-induced azo dye metabolism resembles a causal ambiguity that needs to be clarified.

Although the dysbiotic role of these dyes on gut microbiota have garnered recent attention, limited data are available to highlight the mutual impact between gut microorganisms and these food coloring agents. It was reported that consumption of Allura Red AC causes dysbiosis in human adults along with headache, and hypertension [10], and recently, tartrazine has been associated with gut microbiota dysbiosis in juvenile crucian carp fish [11]. Moreover, ingestion of Allura red AC with high fat diet led to dysbiosis and low-grade colonic inflammation in mice [12].

Due to the scant data around the dysbiotic effect of these azo dyes, our recently published study makes a significant contribution to the emerging field of toxicomicrobiomics. We examined the impact of chronic Sunset Yellow administration on the composition of the rat fecal microbiome and revealed a marked alteration in the gut microorganisms compared to the normal rats [13]. We also reported that the specific effects of Sunset Yellow on gut microbiota are distinct leading to decreased species richness and diversity. Notably, Sunset Yellow chronic administration led to significant reductions in certain taxa, including the Spirocheatota phylum and genera such as *Anaerobiospirillum* and *Helicobacter*, while increasing the abundance of others, such as *Prevotella* 2 and *Oribacterium*. These changes may be attributed to the metabolism of Sunset Yellow by gut bacteria, resulting in the production of metabolites with potential toxic effects. The exclusive presence of certain genera in the Sunset Yellow-treated group, such as *Bilophila* and *Mailhella*, highlights the unique microbial composition associated with Sunset Yellow administration.

Azo dyes trigger inflammation: A microbiota perspective

The interplay between dysbiosis, the azo dye coloring agents, and inflammation and their impact on human health has been highlighted recently [14,15]. Alteration of the gut microbiota composition by food additives has the potential to influence various species such as *Firmicutes*, *Bacteroidetes*, *Barnesiella*, *Prevotella*, *Ruminococcaceae*, and *Bifidobacterium* [16]. These microbiomes are essential for maintaining gut barrier integrity, metabolizing short-chain fatty acids, decomposing fibers and sugars, and are associated with conditions such as obesity, diabetes, and inflammatory bowel disease. In this respect, Allura Red AC was found to trigger intestinal inflammation and to induce colitis, particularly by dysregulating interleukin-23 [14]. This verity was further supported by our data, which reported a crosstalk between microbial changes and intestinal inflammatory responses, as well as disruptions in gut barrier function [13]. To further understand the consequences of gut inflammation caused by these coloring agents, Chen et al. [17] stated that consumption of Allura Red AC induced colitis in mice and triggered inflammatory mediators such as IL-23 and interferon-gamma (IFN-γ)+ cytotoxic CD4+ T cells leading to the apoptotic cell death of intestinal epithelial cells. These findings indicate that the immune system protects the body against harmful stimuli by triggering a complex inflammatory response to defend against infections. However, this defense may result in cell death, a fact that is not extensively addressed in research on azo dyes.

In addition to apoptosis, overexposure to food additives and environmental pollutants can trigger cell death through pyroptosis, another form of programmed cell demise elicited by proinflammatory signals. This process has been strongly associated with the pathophysiology of various diseases [18,19]. Our study further validates this observation, where administration of Sunset Yellow to male Wistar rats for 90 days resulted in the death of intestinal epithelial cells via pyroptosis, accompanied by inflammation and the dysbiotic effects of Sunset Yellow [13].

Inflammasome Activation and Pyroptosis: Key Mechanisms in Inflammatory Cell Death

To clarify the link between dysbiosis and inflammasome/pyroptosis pathway, it is recognized that the altered microbial environment can activate what is known as pattern recognition receptors (PRRs). These receptors are proteins capable of recognizing molecules commonly connected to pathogens and/or altered tissue components identified as pathogen-associated (PAMPs) and damage-associated (DAMPs) molecular patterns. The engagement to PRRs

triggers co-stimulatory signals that activate inflammatory events in adaptive immune cells [20], ultimately leading to pyroptosis. This type of cell death is characterized by excessive cellular swelling, followed by rupture and the release of cellular constituents, thus initiating strong inflammatory response [21]. The pyroptotic trajectory comprises both the canonical pathway, which depends on activated caspase-1 through an inflammasome assembly, and a noncanonical route, which relies on caspase-4/5/11 activated independently of the inflammasome [22]. The non-canonical trajectory can induce pyroptosis upon sensing the bacterial endotoxin lipopolysaccharide (LPS) [23,24]. Although LPS is considered one of the PAMPs, it also activates these caspases by binding to their N-terminal caspase-activation and recruitment domain (CARD) which mediates LPS recognition and oligomerization [24].

For the canonical arm, the Nod-like receptor (NLR) family pyrin domain (PYD)-containing 3 (NLRP3), which is among the well-known inflammasome scaffolds, must be activated by sensing ample exogenous and endogenous microbial metabolites or byproducts, including LPS. Activation of NLRP3 recruits the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC), leading to its polymerization [25]. These inflammasome platforms then activate caspase-1, which stimulates the maturation of the proinflammatory cytokines pro-IL-1β and IL-18 into their active forms and trigger pyroptosis through the activation of caspase-1 and -11.

Notably, the gene encoding caspase-1 is adjacent to that expressing caspase-11 on the same chromosome. In contrast to caspase-1, which requests an assembled inflammasome for activation, caspase-11, representing the non-canonical hinge, can be directly stimulated by recognizing the bacterial byproduct LPS to induce pyroptosis [24]. Upon activation by danger stimuli, the N-terminal of gasdermin D (GSDMD), which is now designated as the chief executioner of pyroptosis, is cleaved by inflammatory caspases [26,27]. The N-terminal fragment then attaches to membrane lipids, causing the disruption of cellular membrane components and forming pores that facilitate osmotic cell rupture and the release of cellular contents.

The expression of the gasdermin protein family in the gastrointestinal tract proposes their fundamental role in the mucosal barrier machinery. In addition, cell rupture caused by GSDMD releases various DAMPs, further exacerbating inflammatory and pyroptotic events through recognition by PRRs. At this critical juncture, pyroptotic cells are forced to undergo an irreversible and disruptive fate.

The altered microbiome following the consumption of Sunset Yellow contributes to the release of LPS, initiation of inflammasomopathy, and pyroptosis, in addition to loss of the intestinal integrity as revealed in our article [13]. This fact has been previously documented, where pyroptosis was triggered by several microorganisms, including viruses, fungi, and bacteria [28-30]. By the same token, chronic consumption of Sunset Yellow increased the abundance of both the *Prevotella* genera and the *Prevotellaceae* family while decreasing the abundance of the *Ruminococcaceae* family. These changes were correlated with activated NLRP3 to be consistent with recent findings reported by Wu et al. [31] in pregnant women and Xue, et al. [32] in a rat model of ulcerative colitis.

Conclusion

In brief, the impact of azo dyes on dysbiosis and intestinal inflammation underscores the intricate relationship between environmental factors and gut health. Through disrupting the balance of microbial communities within the gut, azo dyes have been implicated in promoting dysbiosis, which in turn contributes to intestinal inflammation. As evidenced by the growing body of research, understanding, and mitigating the effects of azo dyes on gut microbiota are crucial steps towards addressing gastrointestinal disorders and promoting overall health. Continued investigation into the mechanisms underlying these processes is essential for developing targeted interventions and public health policies aimed at minimizing the adverse effects of azo dyes on gut health and well-being.

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