

# Gut Microbiota and Serotonin - Biochemical Pathways in Age-Related Macular Disease

CRISTINA DANCA<sup>1</sup>, CLAUDIA FLORIDA COSTEA<sup>1,2\*</sup>, VICTOR VLAD COSTAN<sup>3\*</sup>, MIHAELA DANA TURLIUC<sup>4,7</sup>, ANCA SAVA<sup>5</sup>, SERBAN TURLIUC<sup>6</sup>, ANDREI CUCU<sup>7</sup>, RALUCA ALINA DRAGOMIR<sup>3</sup>, IOANA SADIYE SCRIPCARIU<sup>8</sup>, NICOLETA DUMITRESCU<sup>9</sup>, ALEXANDRU CARAULEANU<sup>8,10</sup>

<sup>1</sup> 2 nd Ophthalmology Clinic, Prof. Dr. Nicolae Oblu Emergency Clinical Hospital, 2 Ateneului Str., 700309, Iasi, Romania

<sup>2</sup> Grigore T. Popa University of Medicine and Pharmacy, Department of Ophthalmology, 16 Universitatii Str., 700115, Iasi, Romania

<sup>3</sup> Grigore T. Popa University of Medicine and Pharmacy, Department of Oral and Maxillo Facial Surgery, 16 Universitatii Str., 700115, Iasi, Romania

<sup>4</sup> Grigore T. Popa University of Medicine and Pharmacy, Department of Neurosurgery, 16 Universitatii Str., 700115, Iasi, Romania

<sup>5</sup> Grigore T. Popa University of Medicine and Pharmacy, Department of Anatomy, 16 Universitatii Str., 700115, Iasi, Romania

<sup>6</sup> Grigore T. Popa University of Medicine and Pharmacy, Department of Psychiatry, 16 Universitatii Str., 700115, Iasi, Romania

<sup>7</sup> 2 nd Neurosurgical Clinic, Prof. Dr. Nicolae Oblu Emergency Clinical Hospital, 2 Ateneului Str., 700309, Iasi, Romania

<sup>8</sup> Clinic of Gynecology, Cuza Voda Hospital of Obstetrics and Gynecology, Iasi, Romania

<sup>9</sup> Grigore T. Popa University of Medicine and Pharmacy, 4 rd year student, 16 Universitatii Str., 700115, Iasi, Romania

<sup>10</sup> Grigore T. Popa University of Medicine and Pharmacy, Department of Obstetrics and Gynecology, 16 Universitatii Str., 700115, Iasi, Romania

*Age-related macular degeneration (AMD) is a multifactorial disease and is a major cause of blindness in the entire world. Current studies show a tight connection between gut microbiota and AMD. This literature review shows the positive role played by gut microbiota, which is essential in providing the optimal serotonin level in retina protection against the noxious action of various factors of the environment. Serotonin or 5-hydroxytryptamine (5-HT) is a monoamine neurotransmitter derived from tryptophan which is poorly expressed in the retina, but it may provide protection against retinal damage, such as light-induced retinopathy and age-related macular degeneration, due to the fact that certain serotonin receptor agonists and antagonists of those 7 classes and 17 subtypes of 5-HT receptors help prevent serum deprivation, anoxia and oxidative damage. Our study also shows the role played by other diet-related factors, which protect retina against oxidative stress and delay the onset of AMD.*

**Keywords:** age-related macular degeneration, microbiota, serotonin, inflammation

Clinical and biochemical data mention oxidative stress in the pathogenesis of age related macular degeneration (AMD). Age-related macular degeneration is the major cause of blindness and visual disability in the elderly [1], along with other eye diseases that may lead to a decrease in visual acuity around the world [2-5]. According to the European Eye Study (EUREYE) [6], an estimated 2.5 million of the European population 65 years and older have AMD and more than 1.1 million have significantly impaired vision due to bilateral AMD. A meta-analysis of population-based data estimated that AMD affects 1.75 million individuals aged 40 years and older in the United States, and owing to the rapidly aging population, this figure is projected to rise to almost 3 million by 2020. AMD is characterized by retinal pigmented epithelial (RPE) cell dysfunction and loss of photoreceptor cells [1,6].

Environmental and demographic factors seem to be involved in the pathogenesis of AMD. Large population-based studies indicated that cigarette smoking and in particular, prior and current smokers are at increased risk for developing AMD, female smokers being at higher risk for progression to advanced AMD, while male smokers may be at higher risk for dry AMD [1]. Obese individuals appear at higher risk for dry and neovascular AMD, while very lean individuals may be at higher risk for dry AMD. Alcohol consumption was not found to be associated with AMD, as reviewed. AMD appears to be more prevalent in the white population [7]. Among demographic factors, the female sex, light skin pigmentation and light iris pigmentation may represent risk factors for AMD. Among environmental factors, excessive visible light exposure may

be linked to the risk of developing AMD. Improving knowledge of the multiple genetic, environmental and demographic factors involved in the pathogenesis of the dry AMD is expected to enable development of targeted drugs to specifically treat and possibly even prevent the disease.

The microbiome, consisting of microbes and their collective genomes, modulates the host metabolic phenotype and influences the host immune system [8]. Interactions between the gut microbiota and the host immune system begin at birth: the microbiota influences the development of the immune system; and the immune system in turn, shapes the composition of the gut microbiota [9]. Later in life, the gut microbiota also influences immune cell recruitment and initiates inflammation. Crosstalk between the gut microbiota and enterocytes shapes the gut environment and profoundly influences intestinal immune homeostasis [10], which lasts a lifetime. Alterations in the gut microbiota, coupled to increased gut permeability (leaky gut), are widely recognized as relevant to the pathogenesis of several diseases, including autoimmune and neurodegenerative disorders [11].

## Experimental part

### Materials and methods

We performed a comprehensive general review synthesizing data from recent relevant studies. For this purpose we have collected data from the following English language medical electronic databases: Pubmed Medline, Embase and Cochrane Library with no time limit. The

\*e-mail: costea10@yahoo.com; Phone +40744972648; victorcostan@gmail.com; Phone +40743118228

present article reviews new data from epidemiologic studies, about the role of gut microbiota and some factors that are positively involved in the protection of the retina. Our aim was to verify if there is any factor in our lifestyle linked to our diet, that is able to protect the retina of oxidative stress. Being known that a healthy microbiota is essential for optimum serotonin level, we identified some issues that are correlated with the level of inflammation in the retina and the answer of our immune system.

## Results and discussions

### *The impact of serotonin on the retina*

The serotonin or 5-hydroxytryptamine (HT) is a monoamine neurotransmitter.

Biochemically derived from tryptophan [12], serotonin is primarily found in the gastrointestinal tract, blood platelets, and the central nervous system of animals, including humans.

In RPE of late stage AMD patients, the levels of antioxidant enzymes are elevated, suggesting a defensive response to increased oxidative stress [13].

The 5-HT<sub>1A</sub> receptor is a potential target for controlling oxidative stress in the retina and RPE. Activation of this receptor is coupled to survival signaling in both neuronal and non-neuronal cells [14]. The 5-HT<sub>1A</sub> receptor is expressed in the neural retina and the RPE.

AL-8309B (Tandospirone, Alcon Laboratories) (1.0% and 1.75% ophthalmic solution) is a selective agonist of the serotonin receptor (5-HT<sub>1A</sub>) that has been shown to protect the retina from severe photo-oxidative stress [15]. AL-8309B was previously reported to upregulate antioxidant defense mechanisms in the retina [16] and to interfere with the complement pathway, preventing the deposition of complement C3, factor B, factor H, and membrane attack complex (MAC) [17]. AL8309-B was evaluated in a randomized, double-masked, multicenter, placebo-controlled Geographic Atrophy Treatment Evaluation (GATE) clinical study, as a topical ocular treatment for geographic atrophy (GA) secondary to AMD. No positive results have been reported up until now [18].

Gut microbiota influences the pathomechanism of AMD. Progression of AMD is influenced by single or compounded environmental and genetic risk factors that lead to persistent low-grade inflammation and a largely innate immune response [19]. Evidence for environmental factors predisposing to AMD is supported by the fact that genetically unrelated individuals with shared long-term environmental exposure develop the disease with a concordance of 70.2% [20]. A consequence of cohabitation and common lifestyle habits that prospectively impact disease modifiers such as systemic inflammation is microbial exchange [21]. Given that commensal gut microbiota exert profound influence on digestion, dietary metabolism, endotoxemia, and immune

responses [22] they are prime candidates to impact chronic low-grade inflammation [23].

Microbiota-related low-grade inflammation is characterized by elevated pro-inflammatory gene expression and is a common consequence of an altered host-microbiota relationship caused by instigator bacteria or dietary components that influence intestinal permeability [24]. There is accumulating evidence that asserts the importance of intestinal permeability, a barrier aspect closely associated with the intestinal commensal microbiota as well as with the mucosal immune system, in intestinal and systemic health.

Heightened intestinal permeability can allow for an increased translocation of bacterial products such as lipopolysaccharide (LPS) and other pathogen-associated molecular pattern molecules (PAMPs). These PAMPs impact pro-inflammatory signaling through pattern

recognition receptors (PRRs) of the innate immune system, especially the Toll-like receptors (TLRs) and Nod-like receptors (NLRs), inducing low-grade systemic inflammation. Microglia, perivascular macrophages, a small number of dendritic cells, and RPE cells express various PRRs, including TLRs and NLRs, and may predispose for PAMPs of intestinal origin to impact ocular inflammation [34]. In addition, bacterial products or metabolites from gut microbiota can modulate microglia maturation, morphology, and function [35] and activate retina-specific T cells that are thought to be involved in autoimmune uveitis [36]. Dysbiosis in gut microbes is particularly important for the aging population given that they modulate aging-related changes in innate immunity, sarcopenia, cognitive function, and frailty in general. Here, it was a high interest to evaluate the contribution of intestinal flora to progression of age related macular degeneration in its neovascular form (NV AMD), particularly in the context of obesity-driven choroidal neovascularization (CNV).

Polymorphisms in genes implicated in inflammation predispose to AMD, yet by themselves, no single mutation can account for disease development. To date, the contribution of the microbiome has not been investigated. One study suggests that gut microbiota influences development of neovascular lesions associated with AMD and this is particularly when obesity is a predisposing factor. It was shown that diets rich in fat alter the gut microbiome and in turn elevate choroidal and systemic inflammation and heighten pathological choroidal neovascularization. This effect could originate from increased intestinal permeability to PAMPs secondary to dysbiosis. In this regard, obesity-related changes in gut microbiota have been shown to decrease barrier function of the gut, allowing increased entry of PAMPs into systemic circulation and consequently triggering inflammation. Both antibiotic treatment and microbiotal transplants from regular-chow diet (RD)-fed mice lower systemic and choroidal inflammation, yet only RD-microbiotal transplants significantly lower intestinal permeability. While the microbial community of high-fat diet x regular diet fed mice (HFDxRDT) resembles the one of RD-fed mice more closely than that of HFD-fed mice treated with antibiotics, the effects of neomycin treatment likely occur through a decrease in the absolute number of bacteria present in the gut. Modifying microbiota can reduce systemic and local choroidal inflammation and attenuate pathological neovascularization. Of note, one of the most heavily regulated inflammatory cytokines in an experiment was IL-6. Elevated levels of IL-6 are associated with early and late AMD and are significantly related to smoking, higher body mass index, and *inflammaging*, the low-grade, chronic, systemic sterile inflammation in aging, in the absence of infection.

The impact of serotonin in the relationship of the gut and retina.

Functional integration of urinary and plasma metabolites associated with age-related macular degeneration (AMD) via pathway enrichment analysis showed pathways involved in carbohydrate and amino acid metabolism, as well as oxidative phosphorylation (via the citric acid cycle). Microbial cometabolites whose levels are modulated by the abundance of microbiota, e.g., serotonin, hippurate, trimethylamine, 4-hydroxyphenylacetate, 3-indoxylsulfate, tyrosine, and tryptophan, were highly enriched among AMD features (AMDf) - associated metabolites (enrichment  $P = 6.00 \times 10^{-5}$ ). Furthermore, higher serotonin levels associate with protection against retinal damage in a diet-independent manner, serotonin signals through multiple receptors. Agonists and antagonists of these receptors are neuroprotective against retinopathy.

The associations of microbial metabolites with diet and AMDf suggested that the gut microbiome might be altered by diet. We found a study where the team characterized gut microbiomes in a diet, time, and AMDf context. They separated mice in 3 groups with high glycemia food (HG), low glycemia (LG), and a moderate glycemic index food (HGxLG).

Surprisingly, three parameters of alpha diversity indicated a statistically significant increase in alpha diversity (usually a feature of healthier microbiomes). Together, the convergence of phenotype, diet, and microbiota relationships suggest that the diet-induced change in the microbiota in HGxLG mice also contributes to the arrest of AMDf in these animals. To extract more metabolic insight into the diet-metabolome-AMDF interactions, one study used a semiquantitative network diagram that integrated associations of urinary metabolites, plasma metabolites, and the gut microbial operational taxonomic units (OTUs) to each other within a retinal damage context. Serotonin is among the most central nodes within the network and shows the second strongest association with retina damage based on bivariate correlation analysis. The influence of microbiota is corroborated by the serotonin relationship, because serotonin production derives from tryptophan and is stimulated by gut spore-forming bacteria.

High-glycemia diet as a risk factor for AMD. Recently, we and others observed in epidemiologic studies that, in addition to micronutrients, macronutrient quality [e.g., consuming a diet with a high glycemic index (GI)] is a significant risk factor for AMD onset and/or progress in nondiabetic humans.

The GI appears to be an attractive dietary intervention target, because simple replacement of small amounts of high-index foods (such as white bread) with lower-index foods (such as whole grain bread) can significantly reduce glycemic peaks without requiring a change in overall dietary patterns. However, human tests to show that these epidemiologic data should be translated into clinical practice or exploited to arrest or reverse AMD await more information about and better understanding of the relationship between AMD and the dietary GI. Henceforth, to emphasize the role of carbohydrates in physiologic responses, we use the term glycemia. Americans consume high-glycemia diets, as reflected in the obesity epidemic in this country. Two publications indicate models in wild-type mice demonstrating that the consumption of lower-glycemia diets is associated with delayed AMDf, but an ability to arrest incipient disease was not an objective of these studies, nor were metabolic alterations that explain the glycemia-AMD relations indicated. Increased levels of advanced glycation end products (AGEs), i.e., covalent posttranslational oxidative and nonoxidative modifications of proteins by sugar-derived metabolites, were observed in the mice fed a HG diet. AGEs are cytotoxic and this cytotoxicity is corroborated by the accumulation of AGEs in AMD. Several other age-related diseases, namely diabetes, cardiovascular, neurodegenerative, and inflammatory disease are also associated with accumulation of AGEs, although the diseases are rarely coincident, implying that each has a unique etiology or combinations of etiologies. Having biomarkers and models in which AMDf development and progression can be monitored would facilitate diagnosis and prognosis as well as further understanding of diet-AMD pathomechanistic etiologies.

## Conclusions

Environmental and demographic factors seem to be involved in the pathogenesis of AMD. Improving knowledge of the multiple genetic, environmental and demographic

factors involved in the pathogenesis of the dry AMD is expected to enable development of targeted drugs to specifically treat and possibly even prevent the disease. Gut microbiota influences development of neovascular lesions associated with AMD and this is particularly when obesity is a predisposing factor. It was shown that diets rich in fat alter the gut microbiome and in turn elevate choroidal and systemic inflammation and heighten pathological choroidal neovascularization.

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