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The neurobiology of nutraceuticals combined with light exposure, a case report in the course of retinal degeneration

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ABSTRACT

The present article presents a case report and discusses the neurobiology underlying the potential neuro-repair induced by combined administration of phytochemicals in a patient undergoing photo-bio-modulation (PBM), which improves anatomical and clinical abnormalities in the course of age-related macular degeneration (AMD). After combined treatments the patient with nutraceuticals and PBM had noticeable improvement of retinal tissue with excellent vision for her age and no worsening of corneal guttae, which was present at the time of diagnosis. The present treatment was tailored, based on translational evidence, to improve the autophagy pathway, which is a key determinant in the onset and progression of AMD. In fact, treatment with specific patterns of light exposure combined with specific phytochemicals, may synergize in improving the microanatomy of the retina by restoring its neurobiology. The combination of light exposure, at selective wavelengths, with the effects produced by the intake of specific phytochemicals to treat AMD is reported here as "Lugano Protocol". Such a clinical protocol represents an "in progress" development backed up by translational research. In fact, recent evidence indicates that, specific phytochemicals, when administered in combination may promote anatomical and functional integrity within the retina. These in turn synergize with analogous effects produced by specific wavelengths, when administered at specific time intervals. The synergism between specific light and combined phytochemicals is discussed at molecular level, where recent data indicate how these treatments, when delivered according to specific patterns, may enhance autophagy in the retina. The improvement of retinal morphology and visual acuity, observed in this case report is thoroughly discussed in the light of the key role of autophagy in regulating the integrity of the retinal epithelium. Despite exciting, and consistent with translational evidence, the clinical report of a disease modifying effect during AMD owns the inherent limit of a case report, which requires wide validation in large number of patients. The potential effectiveness of "Lugano protocol" may apply to other types of retinal degenerations, where common alterations in the autophagy pathway do occur. Thus, such a therapeutic approach may extend to a common late stage of retinal trans-synaptic degeneration, where maladaptive plasticity during several types of retinal degenerative disorders eventually converge.

Key words

Photo-bio-modulation • Resveratrol • LuteinVaccinium Myrtillus • Bilberry • Autophagy; Mitochondria • Mitophagy • β-amyloid • Age-related macular degeneration • Retinal pigment epithelium • Drusen

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Introduction

Age-related macular degeneration (AMD) is, at present the major retinal disease, which causes blindness in middle-aged people living in the Western World (Congdon et al., 2004; Pascolini et al., 2004; de Jong, 2006; Jager et al., 2008, Pinelli et al., 2020a, 2020b, 2020c). Despite a great prevalence, the dry variant of AMD occurs most frequently, remaining a disorder with lacks either officially approved treatments or cures. Epidemiological data stratified over time report a growing prevalence of AMD, which is expected to further increase dramatically in the next decades making it urgent to identify potential targets to probe effective treatments (Seddon, 2001; Seddon and Chen, 2004; Datta et al., 2017; Pinelli et al., 2020a, 2020b). This appears to be crucial considering the early stage of the disorder and the feasibility of early diagnosis based on optical coherence tomography (OCT), routinely carried out during eye examination. The chance to improve our insight in the pathogenic steps acting at the onset and progression of AMD is key to plan a disease modifying treatment to halt, and even revert, the course of the disorder before a serious retinal damage occurs. This needs fueling our efforts to produce translational evidence about which determinants in the neurobiology of the retina are key to trigger and sustain degeneration, while suddenly translating non-invasive, harmless remedies to prompt future strategies. Based on these efforts, evidence is growing, which indicates that, autophagy within the retina represents a fine tuner of neuron survival, while it seems to be early impaired, in the course of AMD (Pinelli et al., 2020b, 2020c; Intartaglia et al., 2021). In fact, autophagy is implicated in clearing altered mitochondria and misfolded proteins, which otherwise accumulate in the course of the disorder (Blasiak et al., 2014; Kaarniranta et al., 2020; Nita and Grzybowski, 2020; Bilbao-Malavé et al., 2021; Yako et al., 2021). Misfolded b-amyloid is the classic hallmark of AMD, which tends to aggregate between the RPE and the inner choroid in the form of drusen (Jager et al., 2008; Pinelli et al., 2020c). This associates with a decrease in far and near visual acuity, the loss of contrast sensitivity, color discrimination and occurrence of wavy lines, progressing eventually to blindness (de Jong, 2006; Jager et al., 2008, Pinelli et al., 2020a, 2020b, 2020d). The occurrence of these aggregates progressively recruits the distal pole of the retinal pigment epithelium (RPE) to deposit between RPE and photoreceptors, when autophagy impairment alters the inter-conal (inter-receptor) domain of RPE to constitute the so-called pseudo-drusen (Pinelli et al., 2020c). The class of compounds known as nutraceuticals feature some phytochemicals owning high efficacy as autophagy inducers (Limanaqi et al., 2019, 2020a, 2020b; Pinelli et al., 2020b, 2020c, 2020d; Ryskalin et al., 2020, 2021a, 2021b). Based on this principle in recent studies a potential efficacy of some phytochemicals, such as lutein, resveratrol and bilberry in the course of AMD was assessed (Pinelli et al., 2020a, 2020b). In this recent report the improvement of visual acuity and a long-lasting decrease in the drusenoid area was described at 6-month follow-up in a patient affected by AMD (Pinelli et al., 2020a, 2020c). This approach was based on an in-depth investigation in animal models and AMD affected patients. It is very likely that, these altered molecular mechanisms are key to maintain the homeostasis at the anatomical border between the outer retina, inner choroid and photoreceptors (Pinelli et al., 2020b, 2020c, 2020d, 2021a, 2021b). When studying these mechanisms at preclinical and translational level, emphasis was driven towards a key role played by the autophagy machinery. In fact, this provides a powerful and unique clearing system operating in the retina to remove altered debris containing lipids, misfolded proteins and mitochondria (Pinelli et al., 2020c). Autophagy mostly acts between the retinal pigment epithelium and the choroid Bruch's membrane, where drusen appear to constitute the classic hallmark of AMD (Blasiak et al., 2014; Kaarniranta et al., 2020; Sethna et al., 2021). Nonetheless, even the occurrence of pseudo-drusen, subretinal drusenoid deposit between the RPE and photoreceptors may be induced when altered autophagy occurs with different polarity in the opposite domain of RPE cells between RPE and photoreceptors (Pinelli et al., 2020c; Kim et al., 2021; Shijo et al., 2021; Wu et al., 2021). The occurrence of pseudo-drusen, is postulated to derive from focal subcellular autophagy impairment in the opposite domain of RPE (Pinelli et al., 2020c), and it may serves to distinguish between different phenotypes of AMD (Pinelli et al., 2020c; Kim et al., 2021; Shijo et al., 2021; Wu et al., 2021). In fact, according to the

hypothesis of an exosome–dependent trafficking of misfolded proteins towards different poles of the RPE, as inferred by pre-clinical studies (Pinelli et al., 2020c, 2021a, 2021b), various autophagy-mediated pathogenic mechanisms for AMD may be implicated.

In the common variant classic drusen appear, which strongly depend on RPE dysfunction.

In detail, the updated retinal microanatomy indicates that RPE acts as a pivot to grant outer retinal metabolism (Gass, 1972; Boulton and Dayhaw-Barker, 2001; Bonilha, 2008; Ambati and Fowler, 2012; Kozlowski, 2012). In this way, the sub-cellular organelles and biochemical responses of the RPE to varying insults depending on age may target these cells to produce the onset and progression of AMD. As summarized in Pinelli et al. (2020c) these include: (i) a failure in RPE-dependent retinal protection from oxidative and mitochondrial stress. (ii) A loss of RPE ability to cope with lipid, glycogen, and protein overload. (iii) Impaired renewal by RPE. (iv) A loss of the outer blood–retinal barrier, which is mostly provided by RPE itself. (v) The occurrence of abnormal inflammatory/immune response. (vi) A loss of RPE polarity, thus reverting the metabolic flow towards photoreceptors and from the choroid. (vii) Accumulation via an exosome dependent process (Pinelli et al., 2021a) of extracellular waste material including proteins advanced glycation end products (AGEs) and lipids to constitute the drusen. All these effects appear to be under the influence of the autophagy status within RPE cells.

Therefore, in the present study, we wish to progress the outcome of AMD up to a total reversal of anatomical derangement detectable at OCT by combining different ways to stimulate the autophagy systems. Thus, in search for a potential strengthening of an autophagy-based disease modifying mechanism, a combined therapeutic approach is carried out here, where the very same nutraceuticals were administered in combination, following a natural ongoing stimulus of the retina. The latter represents the natural light, which depending on the specific wavelengths, apart from providing the natural stimulus to generate the process of vision, is able to induce autophagy and it is key in removing mitochondria and altered proteins (Pevna et al., 2021; Stefenon et al., 2021; Yang et al., 2021). We thus probe a treatment based on translational evidence, which combines physical and chemical remedies, such as light exposure and nutraceuticals, according to an amount and time of exposure which is defined here as “Lugano protocol” (Pinelli et al., 2020a) now hypothesized to converge in upregulating the autophagy status within RPE. This approach is carried out in a patient with dry AMD to evaluate to which extent the anatomical and clinical alterations were modified (either worsened or halted, or even reverted) during and after a six months treatment. The molecular mechanisms operating during such a phenomenon are extensively discussed in the light of the interactions at biological level between exposure to long wavelengths and the effects of nutraceuticals to synergize on the autophagy status to counteract the mechanisms generating the neurobiology of AMD.

Methods

Patient presentation

A healthy 82 years old woman undergo an eye visit when she was diagnosed with dry AMD. The patient had surgery in the past for varicose veins and she had hysterectomy, she did not take any drugs but supplements, she never had any eye surgery, she still has her crystalline lens (though the lens is opalescent) along with a slight corrected hyperopia. The patient has corneal guttae (horny guttata) in the form of a dystrophy of the corneal endothelial cells without the stroma or the epithelium being involved and no presence of edema (stage 1).

The opalescence of the crystalline lens was irrelevant to alter the best corrected visual acuity for near and far (BCVA). The patient is affected by allergy to aspirin, and she was reluctant to drug prescription, while willing to take diet supplements. The patient was not suffering from any specific medical disorder.

At the time of the diagnosis of dry AMD she possessed a best corrected visual acuity of 20/30, while the contrast sensitivity was 1.8/2.0. She had wavy lines in the visual field clearly documented as 7 central horizontal lines. The retinal exam revealed central drusen deposition and irregular thickness of the retinal epithelium. The drusenoid area measured 110.000 μm^2.

Treatments

The patient was treated with light exposure supplemented with combined nutraceuticals according to a specific protocol, which is recently published as "Lugano protocol" (Pinelli et al., 2020a). The patient gave her signed informed consent, where the non-invasive nature of the procedure, the risks and the benefits, and potential alternative options for treatment of dry AMD were explained.

During and after the treatment period, the patient was evaluated by using optical coherence tomography (OCT) to assess the occurrence of drusen and the retinal topography was calculated to provide the thickness of the epithelium in various retinal segments. Subjective visual tests were carried out at the same time intervals. Four subjective tests were applied. The Jaeger Chart test and the Snellen Chart test to score the best corrected visual acuity for near and far (BCVA); the Amsler grid test was used to test the occurrence of visual distortion (wavy lines), while the Pelli-Robson Chart test was applied to measure contrast sensitivity. Each test was repeated 3 times in order to express the mean value.

Instrumental and subjective clinical test were carried out at diagnosis (pre-treatment), when the treatment started and following photo-bio-modulation (PBM) or combined PBM and nutraceuticals at 6 and 12 months after combined treatments.

Photo-bio-modulation (PBM)

During the first time interval, the patient was exposed to a total of 9 sessions of light at various wavelengths. Light exposure was carried out during 3 sessions per week during a total time interval of one month. The light exposure consisted of three selective consecutive wavelengths. In detail, PBM was administered through light-emitting diodes (LEDs, Valeda Light Delivery System). Each session consisted of light exposure for a total time of 250 seconds (4 min and 10 seconds) delivered wavelengths of 590 nm, 660 nm, and 850 nm corresponding to yellowish amber red, red, and infra-red, respectively. Light was administered through a beam owing a diameter of 30 nm (nominal) with a direction which was parallel at treatment plane. When the 590 nm light was administered, the power per unit area was set at 65mW/cm^2; for 660 nm light the power per unit area was 8mW/cm^2, while the light beam of 850 nm was applied with a power per unit area of 8 mW/cm^2. Light was administered according to a beam orientation strictly parallel to the treatment plane and horizontal axis. These wavelengths and power per unit areas were combined in four different steps per each session, according to the following pattern. (i) Step 1: 35 seconds of pulsed yellow/amber (590 nm) and infra-red (850 nm) wavelength, with patient's eyes open. (ii) Step 2: 90 seconds of continuous red (660) wavelength, with patient's eyes closed. (iii) Step 3: 35 seconds of pulsed yellow/amber (590 nm) and near infra-red (850 nm) wavelengths, with patient's eyes closed. Step 4: 90 seconds of continuous red (660 nm) wavelength, with patient's eyes closed. Light was administered in 3 sessions per week for 3 weeks during a total time interval of 1 month.

Nutraceuticals

After PBM therapy, nutraceuticals were supplemented in the form of a powder containing equal amounts of lutein from *Tagetes erecta*, resveratrol from *Polygonum cuspidatum* and bilberry, *Vaccinium myrtillus* extracts. Nutraceuticals were administered at the dose of 6 g, daily, 20 days/month, for a total duration of six months. During administration of nutraceuticals the routine diet was not modified otherwise.

Optical coherence tomography (OCT)

The optical coherence tomography is a gold standard exam used in the diagnosis of AMD, which provides a direct visualization and measurement of drusen number and size as well as altered flatness of the retinal surface and derangements of specific retinal layers. OCT consists of a non-invasive imaging procedure, which is based on visible light waves, which are reflected from different layers of the retina and adjacent choroid structures. This method also allows to measure the amount of altered flatness produced by inflammatory exudate and newly formed blood vessels. It allows drusen detection and measurement as well as the calculation of the drusenoid area and volume according to novel protocols (Pinelli et al., 2020d), which most reliably express the engagement of the retina at anatomical level.

Retinal topography

It specifically measures across various retinal segments the thickness of the retinal layer including the inner choroid along the four quadrant of the retina including the macular region. It is useful to detect the specific site where an alteration of the planar arrangement is produced under the mechanical pressure of underlying structures (drusen in dry AMD, exudates and vessels in wet AMD). It is obtained by combining the OCT technique in different axis.

Visual tests

Since as recently published, the measurement of the drusenoid area, even when mostly reliable does not reflect the loss of visual acuity being partially correlated with visual loss (Pinelli et al., 2020d), this needs to be assessed at clinical level. In fact, the occurrence of drusen remains the hallmark of AMD and tells about an ongoing disease process but drusen *per se* do not produce impaired vision (Pinelli et al., 2020d). It is rather the biochemical alteration in the retina, which from one side generates drusen and from the other side alters the process of vision. Therefore, clinical subjective routine test to assess visual ability are mandatory to validate the disease course and potential disease modifying treatments.
Therefore, the patient underwent four subjective tests that measured visual function. The tests were repeated 3 times in order to express the mean value. The tests here used were the following: the Jaeger Chart test, the Snellen Chart test, the Amsler grid test and the Pelli-Robson Chart test (Contrast sensitivity test).

Jaeger Chart test

The Jaeger Chart test was used to determine the near best corrected visual acuity (BCVA). The apparatus consists of a chart reporting short written text blocks of different sizes. The chart is held at a specified reading distance (35 cm) and the patient is asked to read the smallest block of lines she can focus from the biggest block (J10 or 1/10) to the smallest block (J1 or 10/10). If the patient read a specific block of letters without squeezing, that block is considered to be visualized correctly. Different blocks were shown during each detection in order to avoid learning words by heart. In this study the score J10 to J1 is converted in percentage (10% to 100%) where 100% is the maximum visual acuity for near (J1).

Snellen Chart test

The Snellen Chart test was used to determine the far best corrected visual acuity (BCVA). A retro-illuminated wall-mounted Snellen chart is used with the patient standing at 6 m from the chart (Johnson et al., 1998; Chen et al., 2014). The chart includes red and green color bars for an easy and helpful place to start administering the test. There are 10 rows of decreasing size at a pre-determined distance. The patient is asked to read 5 letters per row (from row 1/10 to row 10/10). If the patient read at least 3/5 of letters in a specific row and 1 or 2 letters of the subsequent row, the previous row is considered to be visualized correctly. Different letters are shown during each detection in order to avoid learning letters by heart. Even in this case for measuring far visual acuity, the score 1/10 to 10/10 is converted in percentage (10% to 100%) where 100% is the maximum far visual acuity (10/10).

Amsler Grid test

It is a grid of horizontal and vertical lines used to monitor a person's central visual field. It is a diagnostic tool that, allows detecting visual disturbances caused by changes in the retina, particularly the macula, as well as the optic nerve and the visual pathway to the brain. Amsler Grid test usually helps detecting defects in central 20 degrees of the visual field. The apparatus consists of a white square-shaped grid divided by horizontal and vertical black lines in approximately 20 small squares in each side of the grid. A central black dot is present for orienting the sight. The illumination of the chart is kept steady and optimal to allow the best resolution. The grid is kept at least 33 cm far from the eye. The patient is asked to close one eye and each eye is tested separately. In patients with altered vision the lines of the square appear distorted, otherwise they look parallel (Su et al., 2016). Patients with macular disease typically observe line distortion appearing as wavy, interrupted or disturbed lines with some lines may be missing in the subjective report. In this study, the score ranges from 10 to 1, scoring is given as follows: 10 (0 wavy lines); 9 (1 wavy, interrupted, disturbed horizontal or vertical line); 8 (2 wavy, interrupted, disturbed horizontal or vertical line); 7 (3 wavy, interrupted, disturbed horizontal or vertical line); 6 (4 wavy, interrupted, disturbed horizontal or vertical line); 5

(5 wavy, interrupted, disturbed horizontal or vertical line); 4 (6 wavy, interrupted, disturbed horizontal and vertical line); 3 (7 wavy, interrupted, disturbed horizontal or vertical line); 2 (8 wavy, interrupted, disturbed horizontal or vertical line); 1 (9 wavy, interrupted, disturbed horizontal or vertical line).

Pelli-Robson Chart test

Pelli-Robson Chart test measures the contrast sensitivity defined as the ability to perceive slight change in luminance between regions, which are not separated by clear-cut defined borders. The chart is composed of letters (6 in each horizontal line) arranged in groups, whose contrast varies from high to low. The patient read the letters, starting from the highest contrast, until she is unable to read two or three letters in a single group. Each group has three letters owing the same contrast level, so there are three trials per each contrast level. The score is based on the contrast of the last group in which two or three letters are correctly red. A Pelli-Robson score of 2.0 indicates normal contrast sensitivity, a score of less than 1.5 is consistent with visual impairment and a score of less than 1.0 represents visual disability.

Results

At the time of diagnosis, before any treatment was started, the patient OCT was indicating a dry AMD as reported in Figure 1, with evident drusen occurring both in the macular and extramacular region. The representative tomography indicates a central wide drusenoid area as pointed by the red arrow and red-lined in its horizontal extent. This passed into other abundant drusen in the extra-foveal region both in the medial and lateral segment (Figure 1). Drusen deposition led to a marked alteration in the planar arrangement of the retina with marked irregularities of the retinal pigment epithelium (RPE), (Figure 1). At the time of diagnosis OCT-based retinal topography with map diagram shows a large central drusenoid area (110.000 μm^2), confirming the disruption of the retinal planar arrangement induced by the drusen (Figure 2, red and white arrows), and severe, irregular thinning of the retinal epithelium which mostly affects the foveal region (Figure 2). The variation in the retinal planar arrangement is reported by counts in Table 1, where data report variations in the mean thickness (mm) and volume (mL) given in absolute number percentiles of the retinal epithelium at the time of diagnosis. Strong discrepancies are evident in different retinal fields.

At 6 months after starting the treatment, when 9 PBM sessions, 3 per week were fully carried out a marked morphological improvement of the retinal scanning was evident. As shown in OCT image of Figure 3, a reduction of drusen deposition below the retinal pigment epithelium is evident. In detail, when compared with Figure 1, the drusen still persist both in the macular and extra-macular fields, although the central drusenoid area is much less prominent in altering the planar arrangement of the retina (Figure 3). Such a drusenoid area measured following PBM was reduced from 110,000 μm^2 down to 70,000 μm^2. This is evident in Figure 4 showing retinal topography with map diagram, where the thickness of the retinal epithelium in the central region is closer to the surrounding retina and the discrepancies are attenuated in various retinal fields (Figure 4). This is measured in Table 2 reporting mean numerical values concerning thickness and volume, where variations are markedly reduced.

Following PBM therapy, the patient was administered nutraceuticals (lutein, resveratrol and blueberry) in a 6 g powder daily, 20 days/month, for six months. At 12 months after diagnosis, when of nutraceuticals were fully administered the effects of the add on PBM were evident, with a further remarkable improvement of the retinal scanning. As shown in OCT of images in Figure 5, a suppression of drusen deposition below the retinal pigment epithelium is evident. In detail, when compared with Figure 1 and Figure 3, the drusen are even almost not detectable in single scans and the calculation of the drusenoid area (30,000 μm^2) by computing OCT in the all areas was reduced by almost four-fold compared with the time at diagnosis and it was diminished by more than a half compared to the drusenoid area which was measured following completion of the full PBM schedule. This is confirmed in Figure 6 showing retinal topography with map diagram, where the thickness of the retinal epithelium in the central region is increased similar to the surrounding retina and the discrepancies are attenuated in various retinal fields (Figure 6). This is measured in Table 3 reporting mean numerical

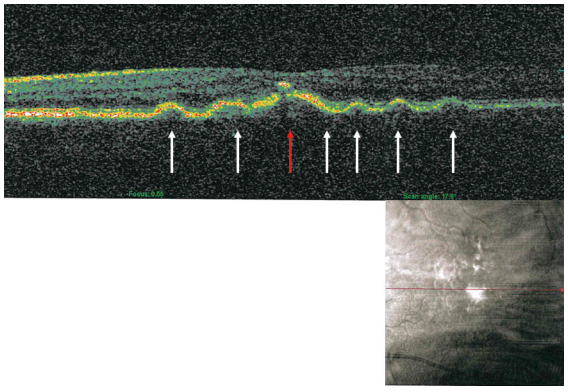


Fig. 1 - Representative Optical Coherence Tomography (OCT) scan from a dry AMD patient at diagnosis (before treatment).
Age-related macular degeneration (AMD) is evident in a female, 82 years old patient with drusen occurring in the foveal region re-lined area pointed by a red arrow and in the extra-foveal region as pointed by white arrows. Drusen deposition associates with an irregularity of retinal pigment epithelium (RPE).

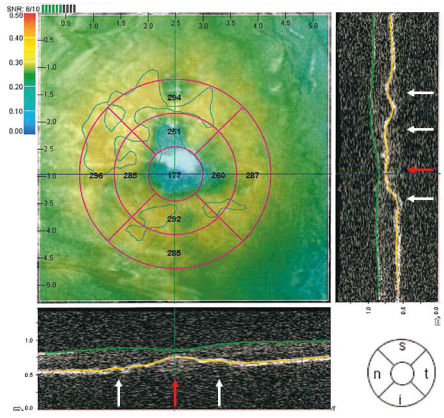


Fig. 2 - OCT-based retinal topography with map diagram in a patient affected by dry AMD at diagnosis (before treatment).
The retinal topography shows a large central drusenoid area (110,000 μm^2). Retinal topography based on OCT detected in the horizontal and vertical axis shows the disruption of the retinal planar arrangement induced by the drusen (red and white arrows), the loss of thickness of the retinal epithelium is severe and mostly affects the foveal region.

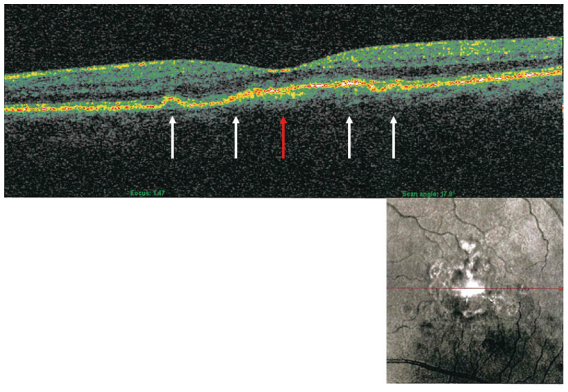


Fig. 3 - Optical Coherence Tomography (OCT) after PBM treatment, at 6 months.
The OCT image after PBM treatment shows a reduction of drusen deposition below the retinal pigment epithelium at macular (red arrows) and extra-macular level (white arrows) at 6 months following 9 PBM sessions, 3 per week.

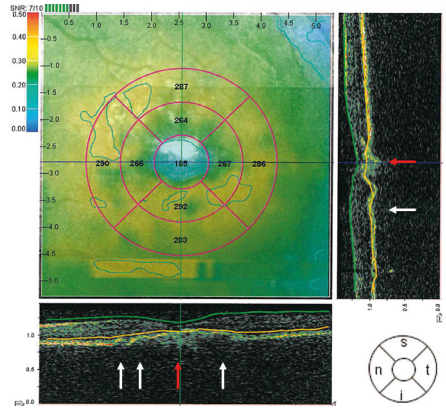


Fig. 4 - OCT-based retinal topography with map diagram after PBM treatment, at 6 months.
The retinal topography shows a suppression of the central drusenoid area (70,000 μm^2). Retinal topography based on OCT detected in the horizontal and vertical axis shows a decrease in the disruption of the retinal planar arrangement induced by the drusen (red and white arrows), the loss of thickness of the retinal epithelium is less severe and this is mostly evident in the foveal region.

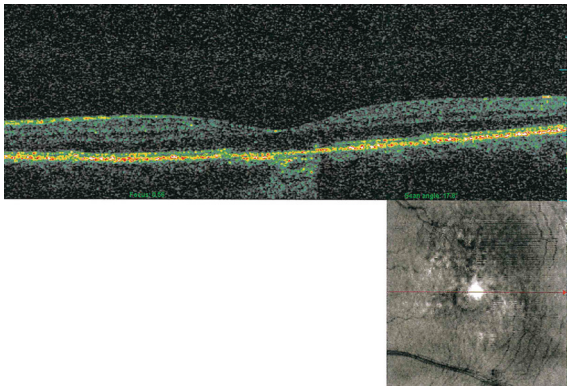


Fig. 5 - Optical Coherence Tomography (OCT) after PBM and nutraceuticals, at 12 months.
The OCT image after PBM treatment shows a reduction of drusen deposition below the retinal pigment epithelium at macular and extra-macular level at 6 months following 9 PBM sessions, 3 per week.

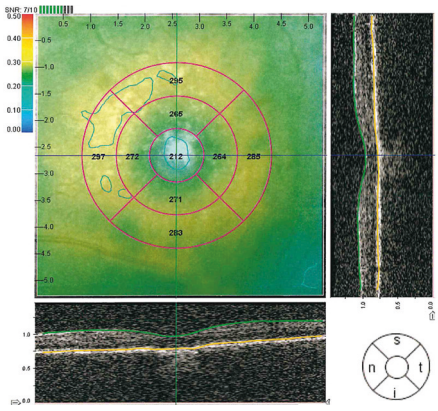


Fig. 6 - Retinal topography with map diagram after PBM and nutraceuticals, at 12 months.
The retinal topography shows a suppression of the central drusenoid area (70,000 μm²). Retinal topography based on OCT detected in the horizontal and vertical axis shows a decrease in the disruption of the retinal planar arrangement induced by the drusen, the loss of thickness of the retinal epithelium is less severe and this is mostly evident in the foveal region.

values concerning thickness and volume, which demonstrates in the central field a two-fold increase in the thickness of RPE compared with the time at diagnosis. At this stage, all values concerning both thickness and volume own a distribution falling within the 95% percentiles (all green in the Table 3, to signify that variability is due to normal discrepancies with a probability higher than 5%, which by definition rules out a disease-dependent effect). At this stage, area and volume variations reported in Table 3 are further reduced and all retinal segments falling into a normal range and being attenuated even in those part which were already in the "green zone" after PBM.

Data obtained by OCT and OCT-based retinal topography were associated with subjective measurement of visual acuity as summarized Table 4, where the progressive improvement of AMD following PBM and further addition of phytochemicals is evident both at clinical and instrumental level. As shown in Table 4, visual acuity was improved by PBM and it was fully re-established following combined PBM and nutraceuticals administration at 12 months as detected by BCVA (Table 4), contrast sensitivity was fully recovered already following PBM at 6 months and it was preserved at 12 months following combined PBM and nutraceuticals (Table 4). Visual distortion as detected by the Amsler test was improved by PBM and it was further ameliorated at 12 months after diagnosis, when phytochemicals were fully administered (Table 4). At this time interval the visual distortion, which amongst visual disturbances most typically correlates with AMD, was suppressed by more than 80% compared with the time at diagnosis. In fact, the wavy lines appearing in the horizontal shapes where distorted very slightly and the amount of distorted lines was greatly reduced. It is remarkable that, such an improvement of visual acuity, specifically detected at the Amsler grid test varies in a way, which overlaps instrumental measurement of AMD-related morphological alterations in the retina detected by OCT and retinal topography. This observation further confirms the validity of the Amsler test in AMD compared with other subjective measurement of visual ability.

Tab. 1 - Thickness and volume of the retinal epithelium at diagnosis.

	Mean Thickness (mm)	Mean Volume (mL)
Center	**94**	
Centre circle	**177**	0.14
Superior inner	**251**	0.19
Temporal inner	260	0.20
Inferior inner	292	0.22
Nasal inner	285	0.22
Superior outer	294	0.40
Temporal outer	287	0.40
Inferior outer	285	0.39
Nasal outer	296	0.41
Totals	275	2.57
Normal distribution percentiles	95% **5%**	**1%**

Data report the mean thickness (mm) and volume (mL) of the retinal epithelium at the time of diagnosis. Strong discrepancies are evident in various retinal fields. The normal distribution of thickness and volume is given in percentiles.

Tab. 2 - Thickness and volume of the retinal epithelium following PBM, at 6 months.

	Mean Thickness (mm)	Mean Volume (mL)
Center	**118**	
Centre circle	**185**	0.14
Superior inner	264	0.20
Temporal inner	267	0.21
Inferior inner	292	0.22
Nasal inner	266	0.21
Superior outer	287	0.39
Temporal outer	286	0.40
Inferior outer	283	0.39
Nasal outer	290	0.40
Totals	274	2.56
Normal distribution percentiles	95%	**1%**

Data report the mean thickness (mm) and volume (mL) of the retinal epithelium at 6 months, following 9 PBM sessions. The discrepancies in various retinal fields are much less pronounced and the normal distribution of thickness and volume is given in percentiles. The thickness is more uniform compared with data obtained at diagnosis (Table 1).

Tab. 3 - Thickness and volume following PBM and nutraceuticals, at 12 months.

	Mean Thickness (mm)	Mean Volume (mL)
Center	181	
Centre circle	212	0.16
Superior inner	265	0.20
Temporal inner	264	0.20
Inferior inner	271	0.21
Nasal inner	272	0.21
Superior outer	295	0.41
Temporal outer	285	0.39
Inferior outer	283	0.39
Nasal outer	297	0.41
Totals	276	2.58
Normal distribution percentiles	95%	

Data report the mean thickness (mm) and volume (mL) of the retinal epithelium following PBM and nutraceuticals, at 12 months following diagnosis. The thickness of various retinal fields is much more uniform compared with Table 1 and Table 2. All data are within the 95% of normal distribution, which reflects the range of normal variability (hypothesis H0 being higher than 5%).

Tab. 4 - Improvement of AMD following PBM and nutraceuticals, at diagnosis, at 6 months, at 12 months.

	At diagnosis, before treatment	At 6 months, after PBM	At 12 months, after PBM and Nutraceuticals
BCVA	20/30	20/25	20/20
CS	1.8	2.0	2.0
Amsler test	Wavy lines (7 central horizontal lines)	Wavy lines (5-6 central horizontal lines)	Slightly wavy lines (2-3 central horizontal lines)
OCT	Central drusen deposition, RPE irregularity	Reduction of drusen deposition	Scarce observation of drusen
Macular topography	Central drusenoid area (110.000 μm^2)	Reduction of drusenoid area (70.000 μm^2)	Further reduction of drusenoid area (30.000 μm^2)

Summary of objective measurement of retinal microanatomy at OCT and macular topography at onset and during AMD following combined PBM and nutraceuticals administration. This is implemented by subjective visual testing showing a recovery from AMD visual symptoms and anatomical alterations which is nearly complete when combined treatments are administered, at 12 months following diagnosis.

Discussion

The present manuscript reports a case of AMD, which greatly benefits from a combined exposure to broad visible wavelengths ranging between amber and near infra-red supplemented by three nutraceuticals, lutein, resveratrol, and blueberry extracts. The benefit was evident in all altered visual symptoms and it was measured by an improvement of deranged retinal anatomy. In particular, it needs to be emphasized how the beneficial effects induced by PBM were further improved by adding a treatment with nutraceuticals. Such a near total recovery should be considered also in the light of disease severity. It is reasonable that the potential plasticity of the retina towards a recovery is likely to be higher when the disease is treated at early stages. On the other hand, the concept about plasticity being much more active in young compared with old people should encourage optimism in considering the advanced age of the patient. We might consider that, despite being 82 years old, the patient suffered from a mild form of AMD, which was likely to be more responsive to disease-modifying treatments. The recovery of integrity is likely to ground on a wider range of functional and anatomical recovery, which exists before the disease progresses downstream in the retina to produce maladaptive plasticity and/or trans-synaptic multiple retinal degeneration.

In fact, the positive outcome of the present treatment, which is known as Lugano Protocol, is more evident at morphological than at clinical level. This is likely to rely on the lower threshold to detect retinal alterations by dedicated retinal morphology. In fact, even at the onset AMD was more evident in the fine anatomy of the macular retina when visual symptoms were still mild. Nonetheless, the improvement in vision was remarkable especially when considering the suppression of visual distortion, which is typical for visual impairment produced by AMD. The suppression of visual distortion is comparable to the suppression of the drusenoid area, being both parameters reduced roughly four-fold following combined treatment with PBM and nutraceuticals. It is remarkable that the improvement produced by PBM as documented by the first post-treatment evaluation at 6 months was frankly expanded by the addition of nutraceuticals. Since prolonged PBM does not appear to exert additional effects, a synergism between retinal stimulation operating according to stimuli pertaining to the physics and chemistry is likely to occur.

In fact, it is not surprising that biological substrates are sensitive to both chemical and physical stimuli mostly when considering the natural exposure of the retina at a gateway of the nervous system with the external environment. The physical energy in the facet of electromagnetic quanta is the natural physical stimulus, which triggers the process of vision. Such a phenomenon does occur based on the ability of light to trigger specific biochemical cascades within the cells of the retina. To make it simple our eyes provide vision since a physical energy in the form of specific electromagnetic wavelengths or quanta interact with specific chemical species to generate the cascade of information, which is needed for the visual processing. It is not surprising at all that the very same physical-chemical interactions, aside from producing a sudden visual effect provide a

trophic activity, which is key in sustaining retinal integrity.

Such a dual effect is typical in the central and peripheral nervous system, where a given neurotransmitter apart from a quick post-synaptic excitation or inhibition also regulates the integrity of the post-synaptic neuron. To make a simple analogy, if we consider a skeletal muscle, we are aware that the phasic action potential in the peripheral nerves produces muscle contraction during a given exercise, while concomitantly affecting robustly the structure of the muscle. It is well-known that, when nervous stimulation to the muscle is protracted and reiterated according to specific time schedules, this provides the best growth and shaping of the muscle cells. This is manifest at gross anatomy and it is detectable at various level of investigation: at subcellular morphology, through the analysis of the fine shaping of cell organelles; at biochemical level by the expression of specific enzymes; at electrophysiology by increased ability to recruit muscle fibers. Accordingly, muscle contraction is modified by the reiteration of nerve activation and so it happens for the baseline muscle tone. Thus, it is not surprising that, the very same stimuli, which operates a quick nervous message also act at delayed time interval to improve the structure and function of a specific target. Coming back to the present case, in the retina, the occurrence of light stimulation is key in maturating the neurons within the retina itself and it strongly contributes to the maturation of a number of neurons within the CNS along the central visual pathways. If one combines the natural physical stimulation of the retina with chemical compounds which are needed to mediate the effects of wavelengths a synergism in promoting retinal integrity is expected. These concepts, which may sound initially as an over-speculation need to be demonstrated to be effective within specific pathways in the retinal cells. To the present purpose this needs to be discussed at the level of those cells which are key in the onset and progression of retinal degeneration in AMD. Indeed, such a concept is firmly grounded by translation evidence. In fact, at the onset of retinal degeneration in AMD, even before the frank occurrence of drusen, a biochemical defect is likely to be present in the RPE (Pinelli et al., 2020c). This seems to be the case of an alteration in the autophagy machinery, which is early impaired in the retina at the onset of AMD (Pinelli et al., 2020c). Such a defect impairs the proper integrity of mitochondria in the retina as well as in the CNS (Guimarães et al., 2003; Fornai et al., 2008; Chertov et al., 2011; Mitter et al., 2012; Natale et al., 2015; Ruffoli et al., 2015; Nita and Grzybowski, 2020; Mastroiacovo et al., 2021; Ryskalin et al., 2021b) and alters the appropriate handling of proteins (Remé, 1981; Remé et al., 1999; Pinelli et al., 2020c; Villarejo-Zori et al., 2021) carbohydrates (Abokyi et al., 2020; Taki et al., 2020) and lipids (Dhingra et al., 2021; Zhang et al., 2021). All these substrates, which cannot be metabolized any further accumulate within drusen. At the same time, a defective autophagy machinery impairs the ability of the RPE to interact with the photoreceptors to handle the pigment turn-over and does no longer enable correct visual processing (Pinelli et al., 2020c). In this way, it is expected that drusen deposition might simply reflect an epiphenomenon where they represent innocent bystanders of a biochemical alteration, which *per se* impairs the visual process instead of being mechanical disruptor of the vision. This concept was recently developed to explain the diaschisis, which often occurs during AMD, between the amount of drusen and visual ability either in a sense or in its opposite (Pinelli et al., 2020d). In fact, based on the advancement we made in the objective measurement of the drusenoid volume and its fractal adjustement to the drusenoid area (Pinelli et al., 2020d) we demonstrated how the occurrence of drusen may develop independently by the loss in visual acuity and, at the opposite, visual acuity can deteriorate even when a few drusenoid area is measured (Pinelli et al., 2020d). Thus, the biochemical defect within the RPE is likely to produce distinct anatomical and clinical effects which, depending on the context may considerably overlap or diverge. These consist in (i) drusen accumulation and (ii) visual impairment. What is counting to improve visual processing is to re-instate the biochemical cascade, which is impaired and it is no longer able to grant a proper functioning of photoreceptors and retinal circuitry. Therefore, dissecting such a pathway and the specific steps being altered is the key to approach AMD. In light of compelling evidence showing a marked alteration of autophagy within RPE in AMD, the effects of phytochemicals should sort beneficial effects. As

a matter of fact, all three nutraceuticals used in the present study are autophagy inducers. This is the case of lutein (Chang et al., 2017; Munia et al., 2020; Pinelli et al., 2020a; Manochkumar et al., 2021), resveratrol (Arena et al., 2021; Brimson et al., 2021; Hu et al., 2021; Odeja et al., 2021; Pineda-Ramírez and Aguilera, 2021) and bilberry (Vaccinium Myrtillus, Haga et al., 2019; Li et al., 2019; Pinelli et al., 2021a). Such an issue was partially explored and discussed in previous manuscripts (Pinelli et al., 2020b, 2020c). This is further confirmed by translational data showing how autophagy inhibition may recapitulate a condition that is reminiscent of AMD (Yao et al., 2015; Zhang et al., 2017; Kozhevnikova et al., 2019). However, in the present manuscript the impressive synergism to improve the course of AMD, which was provided by light in the form of PBM calls for a specific integration in autophagy as synthesized by the following question:

How PBM integrates in the stimulation of autophagy provided by phytochemicals?

In general, by definition PBM involves the use of visible to near infra-red (NIR) light (500-1000 nm) produced by a laser or non-coherent light sources such as light emitting diodes (LEDs) applied to the body to produce beneficial cellular effects. Light in this range penetrates tissue depending on the wavelength and stimulates cellular function via activation of photoceptors (Rojas et al., 2008; Tata and Waynant, 2010; Rojas and Gonzalaz-Lima, 2011). The use of PBM, which was applied in the Lugano protocol includes specifically wide wavelengths ranging from amber light to near infra-red light. If one investigates the effects of these wavelengths on the targets, which are common to nutraceuticals it is remarkable though not surprising at all that autophagy is promoted both by these specific wavelengths as well as the nutraceutical discussed above. For instance, amber light (590 nm) acts on a number of autophagy steps and autophagy molecules. In fact, amber light converts the precursor of the autophagy inducer LC3 I to the active protein LC3 II. Again, amber light produces a higher expression of the autophagy promoter Atg5 gene, it inhibits lysosomal inhibitors leupeptin/NH4Cl and reduces the accumulation of lipid droplets (Choi et al., 2016). In the case of red light, evidence is provided that it reduces misfolded tau protein accumulation and increases inducible HSP70, as well as levels of LC3 II. Similarly, the levels of Atg5 mRNA are significantly increased. This is associated with increased autophagosomes (Commerota et al., 2019; Yang et al., 2021) and augments the autophagy protein Beclin-1 (Stefenon et al., 2021). This recent evidence poses a strong rationale for the synergism between PBM and nutraceuticals in inducing protective autophagy in the course of AMD. An additional point concerns the issue that among these nutraceuticals some are photo-sensitive and can be activated during light exposure. This is expected to occur mostly where light is fully absorbed, which occurs in the pigmented RPE. In fact, the structure and chemical interaction of lutein are modified under the effects of specific wavelength exposure (Aziz et al., 2020), which poses the basis of a molecular interaction where electromagnetic force mutates the biological activities of specific chemical compounds. This is also the case of resveratrol, which synthesis can be increased by light exposure in archaic systems (Lu et al., 2021). This recent evidence suggests an archaic reason for light and plant-derived nutraceuticals to interact with each other with the aim to grant the survival of the system. The light is essential to promote the growth and differentiation in the plants and similar compounds do persist and still interact with light in the human retina to repurpose what it seems to be a fruitful interaction from an evolutionary perspective. In fact, bilberry protects from damage induced by ultraviolet light, while specific wavelengths alter the composition of such a mixture of phenolic compounds by generating alternative metabolites, still acting as autophagy inducers (Karppinen et al., 2016).

Beyond autophagy

The restoration of the autophagy machinery as a common convergence between light and nutraceuticals in the process of modifying the course of AMD does not rule out alternative mechanisms where chemical and physical stimuli may synergize. For instance, the ability of both stimuli to activate a number of retina stem cells niches should be considered. In fact, in the adult eye, various stem cell niches are described were specific wavelengths and nutraceuticals compound are expected to synergize to provide retinal restoration. These adult niches

include a number of areas. In humans and rodents different regions of the adult retina such as the RPE itself (Salero et al., 2012; Bernstein et al. 2020), Müller glial cells (Das et al., 2006; Bhatia et al., 2010; Singhal et al., 2012; Zhao, et al., 2014); the area between ora serrata and terminalis where the ciliary epithelium occurs (Coles et al., 2004; Das et al., 2006; Nickerson et al., 2007; Bhatia et al., 2009, 2010; Aladdad et al., 2019), the iris pigment epithelium (Haruta, et al., 2001; Seko et al., 2012) and the areas of the optic nerve (Bernstein et al., 2020) possess cells characterized by varying degrees of stemness. Among them, the ciliary epithelium and Müller glia are identified as two main retinal stem cell sources. Guo et al. (2021) recently demonstrated the effects of light exposure on stem cells in the CNS. In their study near infra-red light of 808 nm is neuroprotective and activates hippocampal stem cells when applied at 20 mW/cm^2. It is likely that such an effect is translated towards hippocampus via extra-geniculate retinal pathways. If this is the case, the activation of resident stem cells in the retina may occur easily right where the light impacts following such a pattern of PBM. In fact, direct induction of stemness in astrocytes exposed to light was recently shown by Yoon et al. by using red light (Yoon et al., 2021) and the proliferation of stem cells in the inner ear can be induced by infra-red light (Chang and Lee, 2021). This is expected to have a profound impact on eye stem cells, where a concomitant activity of nutraceuticals was recently hypothesized (Pinelli et al., 2021a).

Conclusions

Here we describe a case report, which suggest that specific nutraceutical compounds may exert beneficial effects on the progression of dry age-related macular degeneration (AMD), an eye disease with no approved treatment or cure. The effects of specific phytochemicals as autophagy inducers is well known both in the central and peripheral nervous system (CNS and PNS, respectively). In detail, specific phytochemicals such as lutein, resveratrol and Vaccinium Myrtillus, are all powerful inducers of the autophagy machinery and their protective effects are enhanced when PBM is administered at specific wide spectrum light according to the Lugano protocol. The case report was planned based on the synergism between these nutraceuticals and specific wavelengths to stimulate the autophagy pathway. In fact, in AMD a defective autophagy activity occurs, which is likely to be responsible both for loss of vision and drusen accumulation. Accordingly, the patient developed a marked improvement in visual acuity with a near total disappearance of drusen.

The strength of the present manuscript consists in reporting a protocol, which is based on neurobiological evidence of synergistic enhancement of two autophagy-stimulating approaches. Alternative explanations for such a synergism may concern the ability of light to make these photosensitive compounds more effective. Finally, the chance that a combined stimulation of specific niches of adult retinal stem cells may occur under the effects of light and nutraceuticals should be investigated.

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