CHAPTER II

LITERATURE REVIEW

Age-related macular degeneration

Age-related macular degeneration (AMD) is a vision disorder caused by abnormalities of the retina area called the macula. This disease is a leading cause of irreversible blindness in people over the age of 60 [1]. When central vision is affected, daily tasks such as reading, writing, and driving, can be impaired. Nowadays, the prevalence of AMD is likely to rise as a consequence of increasing longevity [2]. Therefore, AMD will have enormous social and economic impacts on the health care system. Ongoing and future preventive or therapeutic strategies designed to slow the rate of the progression of AMD are therefore received much attention from scientists.

Anatomy and pathophysiology

The center of the retina called the macula is responsible for fine visual acuity and color vision or central vision. The remainder of the retina provides peripheral vision and dark adaptation. The macular function is lost by the degenerative changes of aging.

The macula is an area up to 5.5 mm with the fovea at its centre. The centre of fovea is thinnest part of retina. This area is free from blood vessels and is referred to as the capillary free zone. The macular has predominance of cone cells and is responsible for detailed central vision. The retina is a complex multilayered structure consisting of two parts. The first part is the photosensitive layer of rods and cones and their neural connections that convert light to electrical nerved impulses. The second part is retinal pigment epithelium and basal lamina called Bruch's membrane, functioning to maintain the barrier between the choroid and the retina. The choroid which is mainly a vascular tunica is sandwiched between retina and the sclera.

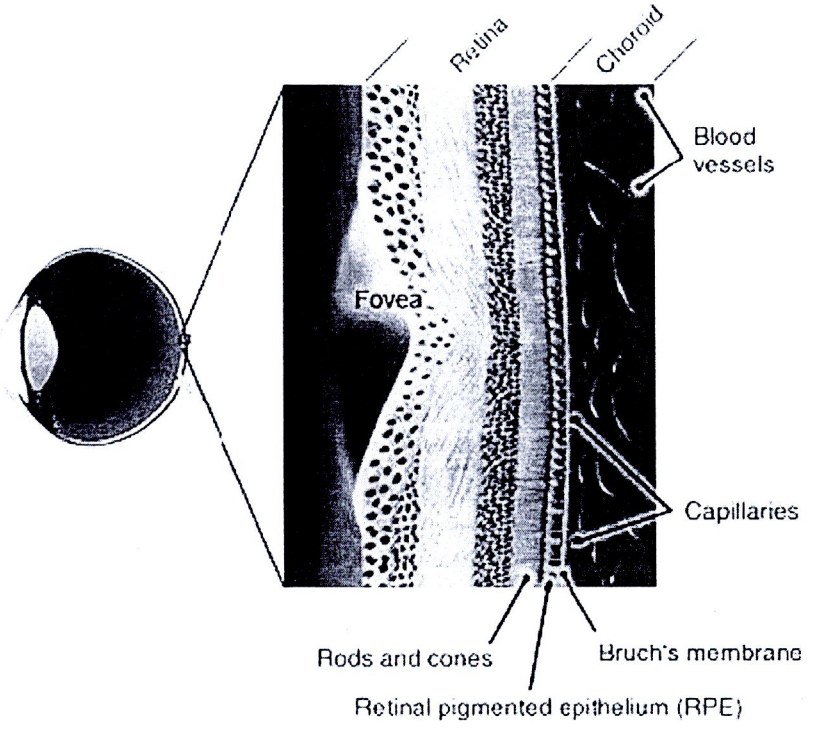

Figure 1 The central area of the retina responsible for fine, detailed central vision.

Source: http://www.eyedrd.org/2011/02/retina-specialty-what-does-a-retina specialist-do.html

Retinal pigment epithelial cells

The retinal pigment epithelium (RPE) is a monolayer of pigmented cells forming a part of the blood/retina barrier [25]. The apical site of the RPE faces the photoreceptor outer segments whereas the basolateral site faces Bruch's membrane. Long apical microvilli surround the light-sensitive outer segments. The functions of RPE cells are absorbing the light energy focused by the lens on the retina [26] and transporting ions, water, and metabolic end products between photo receptor and the blood [27]. RPE cells are essential to visual cycle and retinoid cycles [28]. RPE cells also maintain the phagocytosis of shed photoreceptor outer segments [29] and secrete a variety of growth factors for controlling the structural integrity of choriocapillaris endothelium and photoreceptors. [30, 31, 32]

Etiology of age-related macular degeneration

The hypothetical of pathogenic events for pathogenic AMD includes genetic defect, RPE and Bruch's membrane senescence, ocular perfusion abnormality, and oxidative insult [33].

A. Genetics

Several genes were found to correlate with genetically different forms in AMD pathogenesis. These genes include (1) manganese superoxide dismutase gene [34], (2) CST3 gene is a specific gene that codes for cystatin C, an inhibitor of the cathepsin enzyme, (3) angiotensin converting enzyme (ACE) gene [35], and (4) ABCR gene at chromosome 1p21, a rod-specific ATP-binding cassette transporter [36].

B. RPE and Bruch's membrane senescence

Senescence refers to the loss of cellular growth potential. The failure of cell proliferation is a result of cell cycle arrest characterized by the inability to proliferate in the present of mitogenic stimuli. Alterations of the senescent of RPE cells include change of gene expression, and shorten of chromosomal telomeres [37], decrease in cellular melanin levels, increase in lipofuscin accumulation [38], loss of their hexagonal shape, and decrease in their density [39]. These changes are thought to contribute to the age-related pathologies of AMD.

C. Ocular perfusion abnormality

There is evidence that the choroidal blood flow is impaired in patients with AMD. The decrease in choroidal blood flow is mainly due to the reduction of choroidal blood volume. The density and diameter of the choriocapillaris are less correlating to the pathological changes of the macula to developed AMD [40]. The abnormality of the choriocapillaris reduces diffusion of waste products derived from the RPE leading to their accumulation in the outer part of Bruch's membrane.

D. Oxidative stress

The most current used AMD pathogenic model is based on oxidative damage that influences the metabolism of photoreceptor and RPE cells [41]. Many age-related pathologies have been attributed to cumulative oxidative damage caused by reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), superoxide anion ($O_2^{\cdot -}$), and hydroxyl radical ($OH^{\cdot}$). ROS arise as by products of cellular metabolism or photochemical reactions. Cell membranes are the main targets of ROS as they contain polyunsaturated fatty acids, whose double bonds are sources of electrons which in turn produces lipid peroxyl radicals. The retina is particularly susceptible to oxidative damage because retina and RPE cells have high O_2 tension [42], tremendous exposure to irradiation, high proportion of polyunsaturated fatty acids in the photoreceptor outer segments [43] and numerous chromophores (lipofuscin, melanin, rhodopsin, and cytochrome c oxidase) [44]. Consequently, RPE phagocytosis of photoreceptor disks could also generate ROS [45].

Risk factor for age-related macular degeneration

A number of risk factors for AMD have been indicated from many epidemiological studies. All suggest that this condition is multifactorial in etiology. These risk factors may be broadly classified into personal and environmental factors. The personal factors may be further subdivided into sociodemographic, ocular, and systemic factors.

Sociodemographic factor

Age is the strongest risk factor associated with AMD. The prevalence, incidence, and progression of all forms of AMD rise rapidly with increasing age [46]. Woman have higher rates for late AMD and soft indistinct drusen than men, whereas retinal pigmentary abnormalities were slightly more frequent in men [47]. The women also have a higher age-specific AMD prevalence than men [48]. Race and ethnicity take part of the different in the prevalence of AMD where it is more prevalent among whites than blacks [49, 50].

Ocular factor

Iris color is a hereditary factor that may be associated with AMD [51]. The darker iris contains more melanin pigment than whiter iris which may provide some

protections to the retina [51]. Macular pigment density plays the potential role in protecting against AMD [52]. The yellow macular pigment, namely lutein and zeaxanthin [53] that act as light scatter and chromatic aberration on visual performance [54, 55], reduce the damaging photooxidative effects of blue light through their light absorption [56, 57], and antioxidant properties [14]. The increase in density of the macular pigment can be achieved by high dietary intake of leafy green vegetables that was associated with a reduced risk of AMD [58]. So the protective effect of macular pigmentation has potential for therapeutic implications.

Systemic factor

Several studies suggested that some diseases can affect risk of AMD, for example cardiovascular diseases. [59, 60], hypertension, [61, 62, 63], atherosclerosis [64], and diabetes [65, 66].

Antioxidant Enzymes

It was found that higher levels of plasma glutathione peroxidase were associated with an increase in late AMD prevalence, not early AMD [67]. However, the biological meaning of this finding remains to be elucidated. The oxidative stress could lead to the induction of antioxidant enzymes, therefore high concentrations of such antioxidant enzymes could be used as indicators of oxidative stress-mediated tissue degeneration.

Environmental factor

The data from several studies provide convincing evidence that cigarette smoking is a risk factor for AMD [68, 69, 70] and related to reduce macular pigment density compared to non smoker [71]. Exposure to sunlight has long been suggested as a risk factor for AMD. Short-term exposures to long-wavelength ultraviolet and blue light can cause retinal damage in animals [72]. There are some similarities between long-term changes seen in laboratory animals exposed to short-wavelength visible light and changes seen in patients with AMD [73, 74].

Nutritional factors

The potential role of nutritional supplements to reduce the incidence or severity of AMD has received a great deal of attention [14, 75]. Some studies have suggested that antioxidants and trace minerals are essential for the proper functioning of some key enzyme systems which then may reduce the risk of AMD [73, 76]

Therapeutic prospects

There is not yet a cure for AMD, but some treatments may delay its progression or even improve vision. Treatments for macular degeneration depend on whether the disease is in its early-stage. The treatments mostly aim at stopping abnormal blood vessel growth. Ranibizumab inhibits vascular endothelial growth factor (VEGF) that is thought to contribute the development of macular degeneration by promoting the growth of abnormal blood vessels in the back of the retina. Laser photo-coagulation is a method using laser light to destroy or seal off new blood vessels to prevent leakage. Photodynamic therapy (PDT) is a method that uses Visudyne, a drug that activated by the laser light, to produce a chemical reaction that destroys abnormal blood vessels.

Supplements used for the AMD prevention.

The high levels of certain antioxidant vitamins and minerals in the retina, and the concentrated carotenoids in the macula have led to the speculation that micronutrient supplementation may confer protection from AMD. Dietary supplement with vitamin C (ascorbic acid), vitamin E (alpha-tocopherol), vitamin A, carotenoids, selenium, and zinc has received a great deal of interest for AMD prevention. Carotenoids include beta-carotene, alpha-carotene, the lycopenes, lutein, and zeaxanthin also have antioxidant activity. Most recommended antioxidants used for AMD prevention are lutein and zeaxanthin because they are normally highly concentrated in the macula and retina. Their antioxidant activity can neutralize free radicals that may be relevant to AMD development.

Lutein and Zeaxanthin

Lutein and zeaxanthin belong to the xanthophyll family of carotenoids and are the two major components of the macular pigment of the retina that responsible for central vision and visual acuity. Lutein and zeaxanthin are the only carotenoids found in both the macula and lens of the human eyes, and have dual functions in both tissues as powerful antioxidants and high-energy blue light filter [77]. The concentration of lutein and zeaxanthin in serum ranged from 0.19-0.79 μmol/L [78]. They are mostly found in high concentrations in dark green, leafy vegetables such as spinach, kale,

collard greens, corn, and egg yolks [79]. Lutein is also the major carotenoid (80% of total caroteniods) found in silk yellow cocoon [80]. In addition to play a vital role in ocular health, lutein and zeaxanthin are important nutrients for prevention of cardiovascular disease, stroke, and lung cancer [81, 82]. They may also be protective in skin conditions attributed by excessive ultraviolet light exposure [83].

Structure

Lutein and zeaxanthin belong to the xanthophylls family of caroteniods both contain hydroxyl groups making them more polar than other carotenoids, such as beta-carotene and lycopene. Although lutein and zeaxanthin are isomer and have identical chemical formula ($C_{40}H_{56}O_2$), they are not stereoisomers. They are both polyiso-prenoids containing 40 carbon atoms and cyclic structures at each end of their conjugated chains. The main difference between them is in the location of a double bond in one of the end rings giving lutein three chiral centers as opposed to two in zeaxanthin (Figure 2).

Source of lutein and zeaxanthin

Plants

In plants, lutein functions as an antioxidant and protects plant cells from photo-induced free radical damage [84]. Blue light is the highest energy form of visible light, and is known to induce photo-oxidative damage by generating ROS. Lutein, with its peak absorption at 446 nm in the visual light spectrum can be screened out blue light, while allowing other wavelengths of light critical for photosynthesis.

Many plant sources contain lutein and zeaxanthin for example spinach, collard green, kale, turnip green, mustard green, broccoli, zucchini, romaine lettuce, green bean, brussel sprout, green pea, cabbage, carrot, green pepper, pumpkin, squash, tomato, cantaloupe, honeydew, kiwi, mango, peach, apricot, tangerine, and corn.

Marigold flower (*Tagetes spp.*), belongs to the Asteraceae family that is well known ornamental plant widespread all over the world with numerous species. The *Tagetes* genus is recognized as a source of natural colors [85] and very interesting biologically active products. The petals of the marigold flower are rich in lutein and

lutein fatty acid esters which represent over 90% of the pigments identified in this plant and generally, lutein is mostly found in the ester form [86].

Silk yellow cocoon

It is well known that caroteniods can be synthesized by all photosynthetic plants and some microorganisms. All animals are incapable of synthesizing them and must acquire them from the diet [87]. The silkworm (*Bombyx mori*) has specific gene encoded an intracellular carotenoid-binding protein which enhances carotenoid uptake [88]. The silkworm can produce pigments containing caroteniods and flavonoids. It was reported that the major carotenoids in yellow silk cocoon was lutein representing 80% of total carotenoids [80, 89]. Carotenoids are transported from the lumen of the midgut to the hemolymph lipoprotein, lipophorin, and from lipophorin into the silk gland [89]. A specific lutein-binding protein (LBP) from the midgut of *B. mori* is identified as carotenoid-binding proteins [90]. Once absorbed, carotenoids are either irreversibly or reversibly modified. Irreversible modification includes (*a*) decomposition of the carbon skeleton into smaller units or (*b*) the addition of new functional groups, *e.g.* hydroxylation. Reversible modification includes (*a*) esterification with long chain fatty acids or (*b*) conjugation with proteins forming carotenoid-protein complexes (carotenoproteins) which are water-soluble and more stable than the carotenoids alone [90].

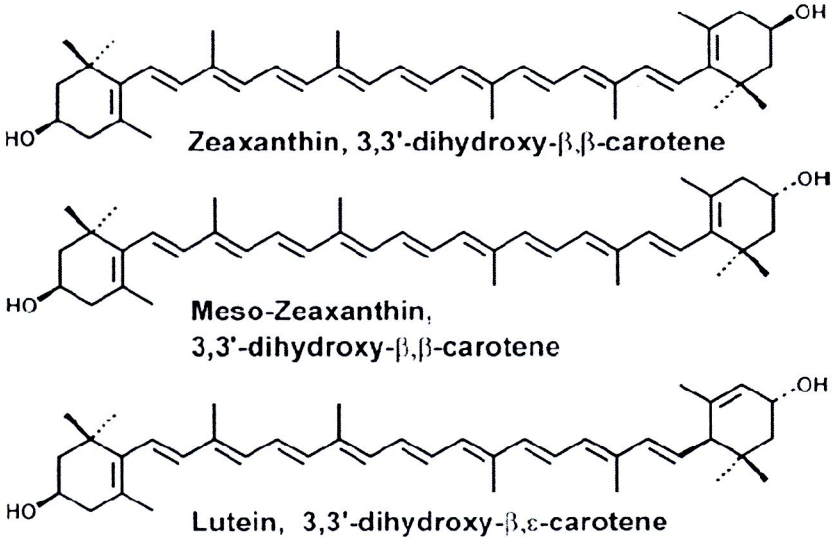

Figure 2 Chemical structure of zeaxanthin, meso-zeaxanthin, and lutein [91]

Physiochemical properties and biochemistry of lutein

The ability of carotenoids to absorb light arises from the presence of a conjugated polyene chain. Lutein and zeaxanthin have nine conjugated double bonds in the polyene chain [92]. Lutein and zeaxanthin differ from other carotenoids in that they each have two hydroxyl groups, one on each side of the molecule. Zeaxanthin is an isomer of lutein, differing only in the location of a double bond in one of the hydroxyl groups. The hydroxyl groups appear to control the biological function of these two xanthophylls [83]. Some dietary lutein appears to be converted to a non-dietary form, meso-zeaxanthin [93]. Infants have more lutein and less meso-zeaxanthin, possibly due to less efficient lutein conversion [94]. Lutein appears to have an affinity for the peripheral retina and rods, while zeaxanthin seems to be preferentially taken up by the cones of the macula [95]. Because xanthophylls are fat-soluble nutrients, their bioavailability is dependent on a number of factors, including nutrient source (whole food or supplement), state of the food (raw, cooked, or processed), extent of disruption of the cellular matrix via mastication and digestive enzymes, and absorption by the enterocytes of the intestinal mucosa (primarily the duodenum).

Pharmacokinetics of lutein and zeaxanthin

Absorption of lutein and zeaxanthin is assumed to follow a similar route of other lipophilic compounds [96]. Lutein and zeaxanthin released from food matrix are digested, conjugated with bile acids or lipids, incorporated into micelles and then absorbed into the mucosal cells of the small intestine via passive diffusion [97]. They are transported from the intestinal mucosa by chylomicrons and are later taken up by hepatocytes, where they are repackaged to lipoproteins and release into circulation [98]. Lutein and zeaxanthin are distributed in high density lipoproteins (HDL) and LDL in fasting blood [99]. Xanthophylls levels in chylomicron increase at approximately 2 h after ingestion and peak blood concentrations were observed at about 16 h postingestion [100]. Lutein and zeaxanthin are major carotenoids found in the blood and account for about 53% of the total caroteniods.

Roles of the action on lutein and zeaxanthin in human health

Lutein and zeaxanthin are powerful antioxidants. Lutein is widely known as the primary nutrient for protecting ocular function. It has long been thought that carotenoids intake also reduces the risk of certain forms of cardiovascular diseases [101], stroke [102], and cancer [103]. It may prevent cellular damage in these conditions by quenching singlet oxygen, neutralizing photosensitizers and inhibiting lipid peroxidation, which then reduce of retinal, cardiovascular [104] and atherosclerosis [81]. Lutein, zeaxanthin, and other carotenoids appear to be depleted in the skin under conditions of prolonged UV light exposure [105]. They also inhibits the generation of ROS-induced inflammation in skin cells, and erythema [106].

Eye protective mechanisms of lutein and zeaxanthin

Antioxidant property

The human retina is particularly vulnerable to oxidative damage because of its high proportion of polyunsaturated fatty acids [107]. High-energy short wavelength visible light and high metabolic activity also promote the generation of ROS which are highly reactive and readily react with lipid, protein and nucleic acids in the macula. It is generally believed that cumulative oxidative damage is in part responsible for the pathogenesis of AMD [41]. The antioxidant properties of lutein and zeaxanthin have been proven mainly based on their abilities to quench singlet oxygen, scavenge free radicals, inhibit peroxidation of membrane phospholipids and reduce lipofuscin formation [108, 109]. They are thought to play a role in the protection against light initiated oxidative damage and appearance of oxidative metabolites of lutein and zeaxanthin within the retina confirming their antioxidant activity in the eye [110]. The properties of xanthophyll carotenoids may affect photoreceptor membrane function by altering permeability, fluidity, lipid phase properties, and the activation of membrane bound proteins [111]. They can reside in the membrane with the hydroxyl groups protruding from the lipid cell membrane into the intra- and extra-cellular plasma membrane, and thus can interact with the ROS inside and outside the membrane [111]. This property makes them highly effective antioxidants. In addition, xanthophyll carotenoids, effectively preserve membrane structure by decreasing the oxygen diffusion concentration products and reducing the rate of chemical reactions with

oxygen which then prevents the fatty acids from lipid peroxidation [112]. Lutein and zeaxanthin appear to have beneficial effects on decreasing the amount of lipofuscin attenuating photooxidative damage of RPE cells induced by lipofuscin [108].

Filter of light

Light exposure leads to retinal damage and increases the rate of photoreceptor apoptosis [113]. Lutein and zeaxanthin absorp light band at 450 nm and therefore these carotenoids can absorb and attenuate the damaging blue light before it reaches the photoreceptors [114]. It has been estimated that macular carotenoids reduce 40% of the amount of blue light reaching to the macula [115]. Therefore, they play important roles as optical filters preventing light-induced photoreceptor damage. The filtering efficacy of lutein and zeaxanthin are superior to those of lycopene and beta carotene [116]. The xanthophylls were incorporated in higher amounts into cell membranes in an orderly orientation, making them ideal optical filters [117]. In membrane, lutein has been reported to act differently in model membranes from zeaxanthin because of the subtle modification at one of its terminal rings [109]. Zeaxanthin tends to lie perpendicular to the membrane surface with the hydroxyl groups in the water phase while lutein appears to have a different orientation in which the hydroxyl group on the terminal ring is either vertical or horizontal to the membrane plane. The two orientations of the lutein molecule allow absorption of light from all directions, thus making lutein have a greater filtering efficacy than zeaxanthin [118].

Lutein and zeaxanthin in AMD

Evidence from epidemiologic studies has shown an inverse relationship between the incidence of AMD and the consumption of diets high in fruits and vegetables containing carotenoids. People ingesting the amounts of carotenoids are the most likely to show evidence of AMD [61, 119]. This same inverse relationship has been observed between the incidence of AMD and serum carotenoid levels [120]. Consumption of diets rich in lutein and zeaxanthin are associated with the low risk for AMD [121]. Supplementation with lutein and zeaxanthin was shown to improve visual function in AMD patients in a double-blind placebo-controlled study of 90 patients known to have atrophic AMD who were followed for 1 year [13]. Results showed that daily supplementation with 10 mg of lutein or 10 mg of lutein plus antioxidant supplementation led to significant improvements in visual acuity, contrast sensitivity, and glare recovery. A significant increase in response in the amplitude densities of the focal electroretinograms was observed in AMD patients taking daily supplements of 10 mg lutein and 1 mg zeaxanthin, suggesting that dysfunction in the central retina could be improved by the supplementation with these caroteniods [122].

Side Effect and Toxicity

No toxicity or adverse reactions have been reported in the scientific literature for lutein and zeaxanthin at doses of up to 40 mg daily for two months [123]. Fijians consume an average of 25 mg lutein daily throughout a life time without any toxic effects [124]. However, high doses of beta carotene supplements (>30 mg daily) have been associated with carotenodermia [125], the same may occur with high doses of lutein and zeaxanthin. Effect of lutein and zeaxanthin in pregnant and nursing women have not been reported, so these women should obtain lutein and zeaxanthin from natural sources of fruits, vegetables, and egg yolks. Ames testing has demonstrated an absence of any mutagenic effect for purified lutein [126].

Ultraviolet radiation

Sunlight is the primary source of radiation reaching the human eye. The solar radiation spectrum ranges from short-wavelength (wavelength 100 nm) to long wavelength far-infrared radiation (100,000 nm). Although UV radiation comprises only 5% of the sun's energy, it carries the most potential for harm. The spectrum of UV radiation ranges from 100 to 400 nm. Ultraviolet radiation is typically classified according to wavelength into the following bands UV-A (400–320 nm), UV-B (320–290 nm), and UV-C (290–100 nm).

Solar UV radiation that reaches to the Earth's surface comprises approximately 95% UVA and 5% UVB. Ultraviolet A radiation exposure produces tanning of the skin and photosensitivity reactions. Ultraviolet B radiation causes sun burning and is the primary "harmful" form of UV radiation. Ultraviolet C is completely filtered by the ozone layer such that it is not found in nature.

The ozone layer acts as a filter to prevent all of UV-C and 90% of UV-B light from reaching the Earth's surface. Over the last 20 years, there has been a depletion of the ozone layer primarily due to industrial pollution. It is estimated that a 1% reduction in the ozone layer leads to an increase in damaging radiation of 0.2% to 2%, primarily UV-B, reaching the Earth's surface [127]. The amount of UV-B radiation reaching the Earth's surface is also dependent upon local environmental factors. The most important feature is the angle of the light rays reaching the Earth's surface. Maximum UV-B exposure occurs in tropical latitudes at midday during summer months. Ultraviolet B levels increase at high elevation by about 10% per kilometer above sea level.

UV-B radiation

UV-B is responsible for the majority of cell damage resulting from direct absorption of UV light by cellular structures. The primary macromolecular structures that absorb UV-B light are DNA and proteins [128]. Other macromolecular structures such as lipids and polysaccharides do not have any major direct absorption bands in the UV-B region. Protein crosslinking and lipid peroxidation are primary photochemical alterations induced by UV-B [129]. UV-B radiation is genotoxic and mutagenic that plays a key role in DNA damage and skin cancer. UV-A radiation is less damaging than UV-B light, but is directly absorbed to some extent by DNA and proteins. The majority of damage induced by UV radiation is resulting from free radical formation and ultimately oxidative damage to DNA, proteins and lipids.

Mechanisms of UV-induced biological damage

UV radiation is capable of inducing biological damage *via* two mechanisms; firstly, direct absorption of UV photons by the cellular material (particularly DNA or proteins) that can lead to photo-induced protein cross-linking, direct DNA damage, dysfunction of enzymes, ion pump inhibition, p53 mutation, and membrane damage [128]. Secondly, possibility of photosensitized processes where UV light is absorbed by an endogenous or exogenous sensitizer that can damage other cellular material. These two major pathways often called Type I and Type II mechanisms. Type I damage involves one electron oxidation or hydrogen atom abstraction from cellular targets, resulting in free radical formation. The Type II mechanism involves energy transfer from the molecule that originally absorbs the UV light (the sensitizer) to molecular oxygen, with the consequent formation of an excited state of oxygen – singlet oxygen. The generated oxygen species are powerful oxidants that can undergo further reactions with cellular materials.

UV radiation and AMD

It has been suggested that AMD is related to the result of repeated oxidative insults and that the outer retina is susceptible to radiation, particularly blue light [130]. The association between sunlight exposure and AMD has been extensively studied but is difficult to prove, because of the difficulty in quantifying ocular exposure to light, especially over a lifetime. There is also the possibility that the susceptibility of the retina to light damage may change with time as aging retina being more susceptible. This could be balanced by increasing browning of the lens that would reduce retinal exposure. There are mixed data on this association between sunlight and AMD. Some study showed a link between blue and visible light exposure (but not UVA and UVB) and AMD [131], and suggested a minimal potential link between sunlight and AMD incidence and progression [132, 133]. This lack of a clear association between UV radiation exposure and AMD could be because the lens absorbs almost all UV-B, and only very small amounts of this wave band can reach the retina. However, one should realize that human can expose in high amount of UV radiation as ozone depletion and global climate changes influence surface radiation levels [22, 134]. Life expectancy increase and lifestyle changes by increasing leisure activities in UV intense environments can also lead to accumulation of UV exposure. Increased exposure to UV radiation has broad public health implications as increased UV related ocular diseases are to be expected.