Cure or cause: opposing roles for zinc in age-related macular degeneration

Expert Rev. Ophthalmol. 3(1),1–4 (2008)

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"Early stages of AMD may be asymptomatic, but later stages involve progressive visual impairment that, in most cases, cannot be treated successfully and the resulting visual loss cannot be restored."

Age-related macular degeneration (AMD) is a chronic degenerative condition. Early stages of AMD may be asymptomatic, but later stages involve progressive visual impairment that in most cases cannot be successfully treated and the resulting visual loss cannot be restored. Early AMD affects approximately a quarter of people over the age of 65 years [1] and late-stage disease accounts for approximately 50% of legal blindness in Europe and North America [2,3]. Recently, genetic factors that put people at risk have been identified (for a recent review, see [4]), but these findings have not yet been translated into preventative measures that would lower the burden of this disease on the affected individuals and their carers.

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The etiology of AMD is largely unknown. The lesions causing visual loss occur as a reaction to age-related changes at the outer retina where the retinal pigment epithelium (RPE), Bruch's membrane (BM) and choroidal vasculature are located. In the early stages of the disease, waste material (identified clinically as drusen) accumulates externally to the RPE, just internally to the choroidal intercapillary pillars in the BM [5]. Drusen in the early stages of the disease do not generally affect vision, but their presence signals altered metabolic state and impaired clear-up function [1]. The pigment of the RPE may also become disturbed with areas of hyper- and hypopigmentation [1]. The presence of drusen deposition and pigmentary changes are easy to identify clinically, and intervention at this stage would be highly desirable. At present, however, there are no medical interventions that could prevent the incidence or progression from the asymptomatic drusen-only stage to end-stage disease with visual loss, largely because of the lack of knowledge regarding why and how early deposit formation occurs. In the majority of cases in the late stages of the disease, the RPE cells slowly degenerate and may atrophy completely. This form of the disease is called 'dry' AMD or geographic atrophy. Geographic atrophy progresses slowly over many years, with the time to legal blindness varying between 5 and 10 years. Currently, there are no treatment options for dry AMD either. If the integrity of BM is broken, choroidal neovascularization occurs, rapidly disrupting visual function. This form of the disease is known as exudative or 'wet' AMD and occurs in approximately 10% of AMD sufferers. Unlike its dry form, wet AMD can be treated with some success (for a recent review, see [6]).

One of the most widely used intervention strategies for AMD is supplementation with zinc. Zinc is often, and wrongly, referred to as an antioxidant molecule. Although it plays an important role in regulating enzymes that are involved in the oxidative processes, the metal itself is redox inert [7]. In combination with vitamin supplementation, zinc was suggested to be beneficial in delaying, arresting or even preventing the development of AMD.

EXPERT REVIEWS

  ISSN 1746-9899

This is a popular option, as antioxidant vitamin and mineral supplements can be purchased without prescription in most countries. However, supplementation trials and epidemiological studies have produced conflicting results. Two Cochrane Reviews have been conducted on the subject. The first review considered randomized trials in which people from the general population had at least 12 months of antioxidant vitamin and/or mineral supplementation [8]. No evidence was found that antioxidants and mineral supplements could prevent or delay the onset of AMD. The lack of benefits of supplements in the primary prevention of AMD was further supported by a recent systematic review and meta-analysis [9]. The data from the second review implied that there may be a modest benefit in taking antioxidant vitamin and zinc supplementation in people with moderate-to-severe signs of the disease, especially in those who have already lost vision in one eye due to wet AMD [10]. While differences in study design, specificity of macular lesions and methods for assessing serum zinc levels and zinc intake made comparisons difficult, the data suggest that the effects of zinc and antioxidant supplementation depend on the stage of disease.

An important question is whether there is a need for zinc supplementation. A healthy balanced diet with a variety of red meat, pulses, fresh fruit and vegetables should provide the necessary vitamins and micronutrients that the body needs. This was proven to be true in a recent large-scale epidemiological study in which adequate dietary intake of vitamins and zinc was shown to be associated with a 35% reduced risk of AMD [11] a better outcome than that of the Age-related Eye Disease Study (AREDS) [12], a supplementation study. Therefore, a regular adequate dietary intake of zinc and vitamins might be all that is needed to reduce the risk of AMD (the recommended dietary allowance for zinc is 12–15 mg/day in the USA and 7–9.5 mg/day in the UK). However, the negative effects of high levels of zinc supplementation are not fully understood. Supplementation with quantities of zinc above the previously suggested upper limit can result in copper deficiency, suppress the immune system, increase the risk for metastatic prostate cancer and impair behavior (for a recent review, see [13]). In addition, increased hospitalizations for urinary complications is present among AREDS patient group [14]. While zinc intake is usually adequate in many communities, there are groups who are at risk of deficiency. Elderly people below the poverty line or people with certain food preferences (e.g., vegetarians or people who only eat fish or chicken meat) are likely to be zinc deficient. This group would certainly benefit from zinc supplements. It is important, however, to consider the bioavailability of zinc from food and supplement sources. On average about 20–30% of zinc is bioavailable in a balanced diet. This, however, is affected by the amount of nondigestible plant ligands like phytates, or some dietary fibres that bind zinc, inhibiting its absorption. The bioavailablity of supplemented zinc is dramatically affected further by its formulation. Zinc oxide, which is the most widely used form in supplements for AMD, has a very low bioavailability, while that of zinc acetate has a is relatively high.

One of the major problems in assessing the need and role for zinc in AMD is the lack of information about its biological role in the retina and surrounding tissues. Like other tissues, the retina can be damaged by too much or too little zinc [6,15]. Newsome *et al.* demonstrated that levels of zinc are reduced in human eyes with signs of AMD [17]. Depletion of zinc increases oxidative stress [18], may cause deficits in phagocytic and lysosomal functions [19,20] and induce macromolecule synthesis- and caspase-dependent apoptosis [15]; mechanisms that are all implicated in AMD. Photic injury is another proposed risk factor for AMD [1] and zinc depletion markedly increases the vulnerability of retinal pigment epithelial cells to UV irradiation injury through UV-induced DNA damage [21]. In addition, inflammation has been associated with AMD [22] and we know that zinc supplementation raises plasma zinc concentration which, in turn, boosts the immune system [23] and provides better protection in AMD and in general aging [24]. Therefore, there are many factors to consider when one opts for zinc supplementation in AMD. While there are many controversies surrounding supplements for AMD there are few other interventions that offer much in the way of disease prevention or cure, so the use of zinc supplements is an important consideration.

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Rather the opposite role for zinc in the pathogenesis of AMD was suggested by our recent findings that drusen, the amyloid plaque-like deposits in degenerating elderly eyes, are filled with anomalous deposits of zinc some of which is 'free' (ionic or loosely protein bound) [25]. We reason that drusen might be formed by a process similar to that driving the formation of plaques in the brains of Alzheimer's patients. While β amyloid peptide, the major component of senile plaques, is present in drusen, the most exciting molecule in relation to drusen and AMD probably is complement factor (CF)H and its *Tyr402His* mutant form [26–30]. Why is this interesting in relation to zinc? Well, almost 30 years ago it was shown that zinc induces the oligomerization of CFH and renders it inactive [31,32], a mechanism that might be potentiated by the introduction of the *Tyr402His* mutation that forms a potential zinc-binding site for further CFH precipitation and inhibition. Although this idea requires experimental proof, modeling studies [33] and our preliminary experiments on CFH support this idea. The inhibition of CFH by such a process may lead to its enhanced precipitation into sub-RPE deposits and the uncontrolled activation

of the alternative pathway resulting in secondary C3 deficiency [34]. The high levels of zinc around the site of sub-RPE deposit formation and in BM in AMD could certainly trigger the precipitation of these, and probably other, proteins. In as much as the evidence implicated drusen as a contributing factor in AMD, then our data suggested that controlling intraocular free zinc might arrest the formation of drusen, and thus macular degeneration, in the aged eye. While it is speculation only, the loss of zinc from retinal cells, described previously, might be the source of the accumulating zinc in drusen, suggesting that damage to pigment epithelium precedes drusen precipitation; an idea that has been debated, but not proven, by earlier studies [1].

As AMD is a progressive disease, there is the potential to intervene in order to limit the amount of vision lost or arrest the disease or even to prevent the disease in the first place. The pathogenesis of AMD is complex and involves multiple genetic, environmental and nutritional factors. The hypothesis that zinc may protect against the disease at the later stages of the disease whereas it may trigger the development of AMD at the early stages is an interesting proposition. However, we do not know at what stage the protective effects of zinc may be important or when the potential negative interactions with genetic and/or other risk factors start. The research to date shows that we cannot extrapolate to taking supplements without good evidence of their safety and effectiveness. If zinc supplementation was demonstrated conclusively to be of benefit, any study would need to investigate the optimal effective dose, for how long that dose should be given and precisely at what stage in the disease process supplementation became effective. Furthermore, potential damaging effects of supplementation, and any relationship to long-term supplementation and dose should be investigated. Most importantly, the role for zinc in the pathogenesis of AMD needs to be clarified as zinc predictably influences many intra- and extracellular functions.

Our finding on zinc, especially on free zinc, in drusen raises the intriguing possibility that a pharmacologic manipulation of the free zinc in the eye might be beneficial against the development of AMD. Zinc-based therapies represent an entirely new approach to pharmacology, because it has only been recognized in the last 10 years that the free zinc ion, like the calcium ion, for example, is an ubiquitous and essential signal ion in biology [35]. In the case of Alzheimer's disease, for example, the seminal discovery was that the amyloid plaques contain zinc that is only very weakly bound to the plaques and is essentially free [36]. This free zinc can be selectively removed from the plaques by compounds that are fairly weak zinc binders [37]. Such compounds are best described as zinc 'buffers', because they do not strip zinc from proteins, but merely stabilize the concentration of free zinc in the milieu. Given the important role for zinc in normal visual processing and its presumed involvement in the degeneration of the retina [38], the goal appears to be the restoration of optimal zinc balance in the eye, which may slow the progression or even prevent the development of AMD.

Financial & competing interests disclosure

The research was supported by Moorfields Eye Hospital Special Trustees and Mercer Fund from Fight for Sight. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Acknowledgements

The authors thank Professor Alan C Bird for helpful comments.

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