Mechanisms of cataractogenesis in the presence of magnesium deficiency

Renu Agarwal¹, Igor N. Iezhitsa¹,², Puneet Agarwal³, Alexander A. Spasov²

¹ Universiti Teknologi MARA, Faculty of Medicine, Level 20, Tower 1, Science & Technology Complex, 40450 Shah Alam, Selangor, Malaysia; ² Volgograd State Medical University, Department of Pharmacology, 1 Pavshikh Bortsov sq., 400131 Volgograd, Russian Federation; ³ International Medical University, IMU Clinical School, Department of Ophthalmology, Jalan Rasah, Seremban, Negeri Sembilan, Malaysia

Correspondence: I Iezhitsa. Universiti Teknologi MARA, Faculty of Medicine, Level 20, Tower 1, Science & Technology Complex, 40450 Shah Alam, Selangor, Malaysia <iezhitsa@yandex.ru>

Abstract. Senile cataract is the most common cause of bilateral blindness and results from the loss of transparency of the lens. Maintenance of the unique tissue architecture of the lens is vital for keeping the lens transparent. Membrane transport mechanisms utilizing several magnesium (Mg)-dependent ATPases, play an important role in maintaining lens homeostasis. Therefore, in Mg-deficiency states, ATPase dysfunctions lead to intracellular depletion of K⁺ and accumulation of Na⁺ and Ca²⁺. High intracellular Ca²⁺ causes activation of the enzyme calpain II, which leads to the denaturation of crystallin, the soluble lens protein required for maintaining the transparency of the lens. Mg deficiency also interferes with ATPase functions by causing cellular ATP depletion. Furthermore, Mg deficiency enhances lenticular oxidative stress by increased production of free radicals and depletion of antioxidant defenses. Therefore, Mg supplementation may be of therapeutic value in preventing the onset and progression of cataracts in conditions associated with Mg deficiency.

Key words: cataract, magnesium deficiency, lenticular ionic imbalance, Ca²⁺-ATPase, Na⁺-K⁺-ATPase, antioxidants

Cataract, i.e. opacity of the lens, is the most common cause of visual disability [1, 2], particularly in the developing countries of Asia [3-6] and Africa [7-10]. As the aging population increases worldwide, the prevalence of senile cataract is also expected to increase. Moreover, several systemic diseases such as diabetes are known to be associated with Mg deficiency and early onset of cataract. Currently, the only established method to restore vision is the surgical removal of cataractous lens and its replacement with an artificial lens. The process involves cost and complications. Several pharmacological approaches have been investigated; however, none has made way to clinical use. In order to devise pharmacological strategies in the future, it is important to recognize and understand the basic pathophysiological mechanisms involved in the onset and progression of cataract.

The lens: structure and homeostasis

The mammalian lens is a living, dynamic structure possessing a unique tissue architecture that allows it to eliminate scattering, and focus light rays on the retina. It is an avascular structure surrounded by a porous connective tissue capsule that has a layer of cuboidal epithelial cells beneath its anterior surface. The epithelial cells at the equator of the lens divide to produce daughter cells that elongate and differentiate into lens fibers that lack the cell organelles. Differentiated lens fibers have a hexagonal shape that allows a compact and homogenous arrangement of fibers. The lens continues to grow throughout the lifetime and the newer fibers are laid down on the top of the existing fibers, which move deeper into the lens. The
mature fibers extend from the anterior to the posterior pole. The lens fibers express in abundance, a soluble cytoplasmic protein known as crystallin that helps to maintain a high refractive index.

**Lens homeostasis: role of the microcirculation**

The maintenance of this unique tissue architecture is vital for the transparency of the lens and for all of the physiological and biochemical events within the lens. As the lens is an avascular structure, passive diffusion is unlikely to be sufficient to support the nutrient supply to the deep-lying fibers, therefore, special transport mechanisms exist in the lens. These mechanisms allow the transport of water, electrolytes and nutrients into the lens fibers and remove from the fibers, products of anaerobic metabolism. Mathias et al. [11] have described a unique microcirculatory system within the lens that maintains lens homeostasis, and therefore, its transparency. According to this description, Na⁺ flows passively from all around the lens, into the lens through extracellular spaces, eventually crossing fiber cell membranes and flowing from cell-to-cell through gap junctions. The gap junctions are highly concentrated in the outer shell of the differentiating fibers in the equatorial region, therefore directing the flow in this direction. The equatorial surface cells contain high concentrations of Na⁺-K⁺-ATPase, which actively transports Na⁺ out of the lens and maintains a high, intracellular K⁺ concentration. Water follows the Na⁺ flux and carries the glucose with it towards the deeper fibers where it is taken up by glucose transporter, GLUT3. The intracellular flow of water occurs through a cytoplasmic major intrinsic protein (MIP) that has strong sequence homology to members of the aquaporin family (AQP) and has been recognized as a major water channel in the lens [12, 13]. Therefore, maintenance of this microcirculation is highly dependent on the functional integrity of Na⁺-K⁺-ATPase.

**Lens homeostasis: role of ATPases**

Besides the Na⁺-K⁺-ATPase, lens fibers, especially in the outer zone, also contain a Ca²⁺-ATPase, which extrudes cytosolic calcium across the plasma membrane or into subcellular organelles in the lens [15]. An Na⁺/Ca²⁺ exchange mechanism also exists in the lens fibers to transport calcium out of the cell [16]. As a result, in the normal lens, the intracellular free Ca²⁺ is in micromolar range as compared to extracellular free Ca²⁺, which is in the millimolar range. Thus, the homeostatic mechanisms in lens that maintain low intracellular Na⁺ and Ca²⁺ and high intracellular K⁺, rely heavily on the functional integrity of Na⁺-K⁺-ATPase and Ca²⁺-ATPase.

**Lens homeostasis: role of redox status**

Like many other organs, the lens is also equipped with antioxidant defense mechanisms to protect cellular structures against oxidative damage and to maintain the structural and functional integrity of cellular components. Intralenticular defenses include non-enzymatic factors such as vitamin C, vitamin E, glutathione (GSH) and carotenoids, and enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase. SOD eliminates superoxide ions by converting them to H₂O₂, while catalase and glutathione peroxidase detoxify H₂O₂. Of them all, GSH is present in an unusually high concentration in the lens and it protects against denaturation of thio-group-containing proteins in the presence of oxidative stress [17, 18] by reacting with H₂O₂ in the presence of glutathione peroxidase and then converting back to GSH under the influence of the enzyme glutathione reductase. Thus, the GSH redox cycle plays a central role in lens antioxidant defense mechanisms, and Mg has been shown to be essential for the activity of enzymes required for glutathione synthesis [19].

**Magnesium deficiency and cataractogenesis**

**Association of magnesium deficiency and cataract**

Although there are no studies focusing on the possible association of primary hypomagnesemia in humans and cataract, indirect evidence from human and animal studies do suggest the possibility of such an association. An association between congenital cataract and a rare autosomal recessive
Mechanisms involved in magnesium deficiency-related cataractogenesis

Magnesium deficiency and lenticular ionic imbalances

Magnesium acts as a cofactor in several key enzymatic reactions. Both the ATPases described above are Mg-dependent. Lenticular total intracellular Mg has been shown to be higher as compared to surrounding aqueous and vitreous humors, particularly so in the peripheral regions [27], which is expected due to the high concentration of Na$^+$-K$^+$-ATPase and Ca$^{2+}$-ATPase in these areas. Apart from these ATPases that use ATP for their functions, several other metabolic reactions that utilize ATP are also Mg$^{2+}$-dependent as ATP in its active form exists in combination with Mg$^{2+}$ as ATPMg$^{2+}$.

In the presence of Mg deficiency, Na$^+$-K$^+$-ATPase functions are lost [28] leading to loss of intracellular K$^+$ and accumulation of Na$^+$ and water leading to cellular damage. Intracellular accumulation of Na$^+$ also causes release of mitochondrial Ca$^{2+}$ by Na$^+$/Ca$^{2+}$ exchange. Loss of function of Ca$^{2+}$-ATPase leads to increased intracellular Ca$^{2+}$. Moreover, due to Mg deficiency there is reduced Mg$^{2+}$/Ca$^{2+}$ exchange at the cell membrane leading to increased intracellular Ca$^{2+}$ [29].

Magnesium deficiency and oxidative stress

A deficiency of Mg has been shown to be associated with increased expression of the inducible nitric oxide synthase (iNOS), an isoform of the enzyme NOS, leading to production of excessive quantities of nitric oxide (NO). Human lens epithelial cells (HLEC), cultured in Mg-deficient medium, were shown to have a six-times higher expression of iNOS compared to those cultured in medium containing normal Mg concentrations. Release of NO was shown to peak at six hours post-incubation. Treatment of medium with iNOS inhibitors, diethylthiocarbamate and aminoguanidine attenuated excess NO release [30]. One of the in vivo studies has shown that treatment of rats with iNOS inhibitor prevented the development of selenite-induced cataract and restored Ca$^{2+}$ and GSH concentrations [31]. Release of excess NO causes cellular oxidative damage by generating nitrogen free radicals such as NO$^+$, NO$^-$, NO$_2$, N$_2$O$_3$, and ONOO$^-$.

The aqueous humor level of NO has been shown to be significantly high in patients with cataract. Moreover, the increase in aqueous NO levels parallels the patient’s age and maturity of the cataract [32]. Lenses of patients with posterior subcapsular cataract show an increased lens concentration of nitrite, a metabolite of NO [33].

In addition to an excessive production of free radicals in the presence of an Mg deficiency, the inadequacy of antioxidant defenses may further contribute to cellular oxidative damage. Studies have shown that ATP depletion due to Mg deficiency causes reduced GSH levels by reducing the glutathione synthetic capacity of cells because ATP and Mg both are required for enzymatic reactions involved in GSH synthesis [34-36]. Reduced GSH levels may also be attributed to its increased utilization due to Mg deficiency-induced oxidative stress [37]. Hsu et al. [38] have shown that Mg deficiency causes GSH depletion and therefore increased oxidative stress.

Lenticular antioxidant mechanisms may also be deficient in the presence of systemic oxidative stress as the regulation of ocular antioxidants such as glutathione depends on its plasma levels [39]. Patients with cataract have shown increased plasma levels of lipid peroxidation products and decreased levels of reduced glutathione [40, 41].
Regulation of erythrocyte glutathione metabolism requires Mg as an essential factor and a deficiency of Mg leads to reduced blood GSH levels. Rats fed with an Mg-deficient diet have shown reduced GSH [38], SOD and catalase [42], and increased lipid peroxidation products [43] and nitrate [44]. Thus, systemic oxidative stress resulting from diseases associated with Mg deficiency may lead to lenticular oxidative stress, which triggers the mechanisms that lead to cataractogenesis.

Mg deficiency and lenticular ATP depletion
Mg deficiency might interfere with ATP synthesis as the mitochondrial reactions that generate ATP require several Mg-dependent enzymes. Human senile cataract lenses as well as lenses from UPL (Upjohn Pharmaceutical Limited) rats, a dominant hereditary cataract model that develops early-onset cataract, have shown ATP depletion [45, 46]. Moreover, excess of NO generated in the presence of Mg deficiency also interferes with several key enzymatic reactions involved in ATP synthesis [47, 48]. HLEC cultured in Mg deficient medium were shown to have significantly low ATP levels, 24 hours post-incubation, as compared to those cultured in medium containing a normal Mg concentration. Treatment of medium with aminoguanidine restores the ATP level indicating the causative role of excess NO production secondary to Mg deficiency [25]. ATP depletion adversely affects the functions of membrane ATPases [49]. As discussed before ATPase functions are essential in maintaining the lens ionic environment. Reduced Ca^{2+}ATPase activity in response to increased iNOS expression and normalization of cellular ATP and Ca^{2+}ATPase function in the presence of aminoguanidine has been observed in UPL rats [50].

End stage mechanism of cataractogenesis in the presence of Mg deficiency
As discussed earlier, the integrity of the soluble cytoplasmic protein, crystallin, is essential in maintaining the transparency of the lens. All of the pathophysiological lenticular changes described in preceding sections eventually culminate in structural damage to crystallin and the development of lenticular opacities. These final steps can be summarized as below:

- Mg deficiency, by causing ATP depletion and dysfunction of membrane associated transporters leads to ionic imbalances, in particular, reduced intracellular K^+ and increased Na^+ and Ca^{2+}.  
- Excess production of NO in the presence of Mg deficiency also predisposes to ionic imbalance by altering the gap junction proteins. Nitrosylation of cysteine residues at the C-terminal domain of connexin46, a gap junctional protein expressed in the lens, has been shown in the presence of excess NO [51].
- Increased intracellular Ca^{2+} causes activation of an intracellular Ca^{2+}-dependent serine protease, calpain. One of the isoforms of calpain, calpain II, is known to cause proteolysis of soluble crystallin forming insoluble products [50, 52-54]. As stated earlier, soluble crystallin is essential for maintaining the transparency of lens and its degradation by calpain II leads to the development of lenticular opacity.
- Nitrite ions have been shown to react non-enzymatically with crystallin leading to changes similar to those seen in senile cataract. Thus, nitration of crystallin as a result of excess NO production due to magnesium deficiency may also be an important mechanism of cataractogenesis [55].
- High intracellular Ca^{2+}, besides causing activation of calpain II, also causes inhibition of another enzyme, acid phosphatase. This enzyme is activated by Mg^{2+} in a dose-dependent manner and is known to play a role in maintaining lens transparency [56].
- The oxidative stress resulting from Mg deficiency is deleterious to cellular function and may lead to lens fiber apoptosis, further contributing to cataractogenesis.

Conclusions
Mg has been recognized to be an important regulator of lens homeostasis. It is required not only for maintaining ion transport across the cell membranes, it is also involved in regulating the cellular redox status. Changes to ATP synthesis and ATPase functions as a consequence of Mg deficiency results in intracellular ionic imbalances triggering the enzymatic reactions that lead to the conversion of soluble cytoplasmic proteins into insoluble forms. Altered redox status within the lens fibers further predisposes to loss of lenticular structure and function. Therefore, reduced lens Mg content may be an important contributor to the onset and progression of cataract in diseases commonly associated with Mg deficiency. Despite
the scientific evidence described in this review, no clinical studies have so far been conducted to investigate the association between primary hypomagnesemia and cataract, and, accordingly, whether or not supplementation with magnesium may have a preventive role in cataract development. There is little reliable information on the comparative importance of magnesium in diet: longitudinal surveys relating diet to progression of cataract are recommended.

Disclosure


References


