Effects of Magnesium Content in the Feed on Cataract Development in Shumiya Cataract Rat

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Abstract: Cataract is a phenomenon in which the eye becomes opaque resulting in severe visual impairment, and senile cataract is the most common cause of blindness in the world. We investigated the effect of magnesium (Mg) supplementation on cataract development using shumiya cataract rat (SCR). The SCR were fed on either a low Mg (Mg 50 mg/kg), standard Mg (Mg 500 mg/kg), or high Mg (Mg 5000 mg/kg) diet from aged 5 to 15 weeks. The growth curve of SCR fed on a low Mg diet was the same as that of SCR fed on a standard diet. The growth curve of SCR fed on a high Mg diet was significantly suppressed in comparison with those fed on a standard diet. The opacification of lenses from SCR fed on a standard Mg diet started at 11 weeks of age. The opacification of lenses from SCR fed on a high Mg diet was similar to that from SCR fed on a standard Mg diet. On the other hand, the low Mg diet accelerated the onset of cataract development, and the opacity started at 10 weeks of age. In addition, the calcium ion (Ca^{2+}) content in SCR lenses fed on a low Mg diet significantly increased in comparison with that in lenses from SCR fed on a standard Mg diet. These results suggest that Mg deficiency causes acceleration of cataract development in SCR, probably due to a rise in the Ca^{2+} content in the lens.

Key words: cataract, magnesium, calcium, Shumiya cataract rat

1 INTRODUCTION

Senile cataract is the most common cause of blindness in the world today¹. The basic mechanism for cataract development is thought to be due to an increase of calcium ion (Ca^{2+}) content in the lens. Elevated Ca^{2+} content in the lens has been induced to activate calpain, a Ca^{2+} -dependent protease. Furthermore, the degradation of lens proteins such as crystallin proteins results in an opaque lens^{2,3}. Cataractous lenses have an altered distribution of the intracellular ionic environment. In human senile cataract, Dilsiz *et al.* reported that lens ionic imbalance with increased levels of Ca^{2+} and sodium ion, coupled with decreased levels of magnesium ion and potassium ion, is related to cataract development⁴. On the other hand, Ornek *et al.* recently reported that the levels of nitrite, a metabolite of nitric oxide (NO), are higher in the lens of human senile cataract patients than in normal lenses⁵. We also reported that induction of inducible nitric oxide synthase (iNOS) protein occurred prior to the increase of Ca^{2+} content in the lenses, and the

administration of an iNOS inhibitor can prevent the elevation of Ca^{2+} content and lens opacification in the Shumiya cataract rat (SCR), UPL rat, and selenite-induced cataract rat^{6,8}. In addition, Yokoyama *et al.* reported that NO production is enhanced by magnesium (Mg) deficiency⁹. Therefore, Mg supplementation may be beneficial in senile cataractogenesis.

In studies to identify preventive measures against senile cataract, the selection of the experimental animal is very important. SCR, which was established by Shumiya and Nagase, is a hereditary cataractous rat strain¹⁰. Lens opacity in SCR appears spontaneously in the perinuclear and nuclear portions at 11-12 weeks of age, and cataract appearance in adult SCR was 66.7%¹¹. Previous investigations have revealed that oxidized glutathione concentrations in the SCR lens are increased, and reduced glutathione values are decreased¹². The proteolysis of some crystallins and cytoskeletal proteins was significantly enhanced in cataractous SCR lenses. The Ca^{2+} contents in

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cataractous lenses rise markedly with age compared with non-cataractous SCR lenses, and the autolytic product of calpain is also detected in cataractous lenses¹³. It is noteworthy that SCR cataracts are not diabetic cataracts. Therefore, SCR is a useful model for studies to reveal the mechanism of senile cataract development and the effect of nutrition.

In the present study, we investigated the effect of cataract development in SCR by a supplementation of diet containing various amounts of Mg.

2 EXPERIMENTAL

2.1 Animals

The rats used were SCRs aged 5 to 15 weeks. They were housed under standard conditions (12 h/day fluorescent light (07:00-19:00), 25°C room temperature) and fed on a powder diet based on American Institute of Nutrition guidelines (AIN-76, standard Mg diet)¹⁴ and water. SCR strain rats were maintained in the Tokyo Metropolitan Institute of Gerontology (Tokyo, Japan). The powder diet consisted of 20% casein, 0.3% DL-methionine, 15% cornstarch, 50% sucrose, 5% cellulose, 5% cornoil, 0.2% choline tartrate, 1.0% vitamin mix and 3.5% mineral mix without Mg. Mg was added as magnesium oxide, and three powder diets were prepared, such as a low Mg (Mg 50 mg/kg), standard Mg (Mg 500 mg/kg), high Mg (Mg 5000 mg/kg) diet. Lens opacity in SCR appeared in exactly 2/3 of animals; the remaining 1/3 had normal clear lenses. In these experiments, the SCRs were divided into two groups, i.e., non-cataractous and cataractous, and were separately housed. The judgment as to whether individual rats would be non-cataractous or cataractous was based on observation with an anterior eye segment analysis system (EAS-1000, Nidek, Aichi, Japan) at 6 weeks of age¹⁵. Animal experiments were performed in accordance with the ARVO Resolution on the Use of Animals in Research.

2.2 Image analysis for cataract development in SCR

The pupils in SCR were dilated by instillation of 0.1% pilocarpine (Santen Pharmaceutical Co., Osaka, Japan) without anesthesia. Changes in the transparency of the lenses were monitored using an EAS-1000 equipped with a CCD camera (Nidek, Aichi, Japan). The outline of the lens image was determined by selecting 4 points on the image and then the transparent area within the outline and thread level were set automatically by the software. The total area of opacity of the lenses, expressed in pixels, was calculated using computerized image analysis software connected to the EAS-1000 system¹⁵.

$\text{Pixels within opacity (Pixel)} = \text{Pixels of cataractous SCR eye} - \text{Pixels of non-cataractous SCR eye}.$

2.3 Assay of Ca^{2+} contents in SCR lenses

Lenses taken from SCR at 15 weeks of age were homogenized in phosphate-buffered saline (pH 7.4) on ice. The lens homogenates were centrifuged at 10,000 rpm for 30 min at 4°C and the supernatant was used for measurements of Ca^{2+} contents. The Ca^{2+} concentrations in the lens were determined according to the methyl xyleneol blue colorimetric method using the Ca test kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and CL-770 (Shimadze Corp., Kyoto, Japan). The Ca^{2+} contents ($\mu\text{mol/g}$) in lenses were expressed as the ratio of the wet weight of the lens.

2.4 Statistical analysis

The data are expressed as the mean $\pm$ standard error of the mean. Statistical difference was evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison. *P* values of less than 0.05 were considered significant.

3 RESULTS

Figure 1 shows the changes of the SCR body weights by diets containing various Mg contents. The growth curve of SCRs fed on a low Mg diet was the same as that of SCRs fed on a standard diet. On the other hand, the growth curve of SCRs fed on a high Mg diet was suppressed significantly in comparison with that of the standard Mg diet, and the body weights at 13 weeks of age were approximately 80% in SCR fed on a standard Mg diet.

Figure 2 depicts Scheimpflug slit images of eyes of cataractous SCR and the effects of diets containing various Mg contents on these, as documented by EAS-1000. Figure 3 shows that the effects of cataract development in cataractous SCRs fed on diets containing various Mg content. During 6-10 weeks of age, the opacity of the lenses from cataractous SCR in this study was similar to that of non-cataractous SCR lenses. The opacification of lenses from cataractous SCR fed on a standard Mg diet started at 11 weeks of age, and mature cataracts had formed at 13 weeks. On the other hand, the lenses from cataractous SCR fed on a low Mg diet started at 10 weeks of age, and mature cataracts had formed at 12 weeks. The opacification of lenses from SCR fed on a high Mg diet was similar to that from cataractous SCR fed on a standard Mg diet.

Figure 4 shows that the Ca^{2+} contents in lenses from non-cataractous and cataractous SCRs, and the effects of diets containing various Mg contents on Ca^{2+} content in SCR lenses. The Ca^{2+} content in cataractous SCR lenses at 15 weeks of age increase in comparison with these in non-cataractous SCR lenses, and the Ca^{2+} contents at 15 weeks of age were about 3-times those in cataractous SCR. In cataractous SCR, the Ca^{2+} content in cataractous SCR lenses fed on a low Mg diet significantly increased in com-

parison with that in cataractous SCR fed on a standard Mg diet.

4 DISCUSSION

In this present study, a low Mg diet accelerated the onset of cataract development in SCR, probably caused by the rise in Ca^{2+} contents in the lens. On the other hand, excessive supplementation of Mg caused a decrease in the growth.

Magnesium ion is a co-factor in many biochemical reactions leading to speculation concerning its role in the etiology of degenerative disease, for example, diabetes mellitus, hypertension, cardiovascular and cerebrovascular diseases¹⁶⁻²¹⁾. In this study, we used a powder diet containing various magnesium oxide amounts for the supplementation of Mg. Magnesium oxide is widely used as an antacid, laxative or Mg supplement²²⁾. Therefore, a decrease in the growth curves in SCRs fed on a high Mg diet was expected. The laxative effect of magnesium oxide may work strongly in SCRs fed on a high Mg diet, resulting in a decrease in the growth.

In the effect of Mg on cataract development, an onset of cataract development in cataractous SCRs was accelerated by the Mg deficiency. The change in opacity levels can be explained as the regulation of the change in Ca^{2+} in the lens, since even in lenses from cataractous SCR aged 15

Fig. 1 Change of the Body Weight of SCR Fed on Various Powder Diets.

Body weights of SCRs were measured from 5 to 13 weeks of age. Low Mg diet fed cataractous SCR (▲), standard Mg diet fed cataractous SCR (●), high Mg diet fed cataractous SCR (■). Data are presented as means $\pm$ S.E. of 5 independent rats. * $P < 0.005$, vs standard Mg diet fed cataractous SCR.

Fig. 2 Scheimpflug Slit Images of Lenses from Cataractous SCR Fed on a Various Powder Diets. Scheimpflug slit images were obtained by an anterior eye segment analysis system (EAS-1000) from 10 to 14 weeks. Standard Mg (Non-cataract), standard Mg diet fed non-cataractous SCR; Low Mg, low Mg diet fed cataractous SCR; Standard Mg, standard Mg diet fed cataractous SCR; High Mg, high Mg diet fed cataractous SCR. The numbers above the photographs show the ages of the rats (weeks).

Fig. 3 Lens Opacity of Cataractous SCR at 10 to 14 Weeks of Age Fed on a Various Powder Diets.

The cataractous SCRs were fed on a various powder diets. Low Mg diet fed cataractous SCR (▲), standard Mg diet fed cataractous SCR (●), high Mg diet fed cataractous SCR (■). The area of opacity (pixels) was analyzed by image analysis software connected to the EAS-1000 from 10 to 14 weeks of age. The data are presented as means $\pm$ S.E. of 10-18 independent rat lenses. * $P < 0.05$, vs. standard Mg diet fed cataractous SCR.

Fig. 4 The Contents of Ca²⁺ in Lenses of Non-cataractous and Cataractous SCR at 15 Weeks of Age.

Open columns, non-cataractous SCR; closed columns, cataractous SCR. Standard Mg (Non-cataract), standard Mg diet fed non-cataractous SCR; Low Mg, low Mg diet fed cataractous SCR; Standard Mg, standard Mg diet fed cataractous SCR; High Mg, high Mg diet fed cataractous SCR. Data are presented as means $\pm$ S.E. of 3 or 7 rats.*1: $P < 0.01$, vs standard Mg diet fed non-cataractous SCR; *2: $P < 0.05$, vs standard Mg diet fed cataractous SCR.

weeks, the increase in Ca²⁺ content in lenses from cataractous SCR fed on a low Mg diet were higher than those of cataractous SCR fed on a standard Mg diet.

We previously reported the involvement of NO in SCR cataract development, and the induction of iNOS protein occurred prior to the elevation of Ca²⁺ content in the lenses⁶⁾. In the human lens, Ornek *et al.* reported that the levels of nitrite are higher in the lens of human senile cataract patients than in normal lenses⁵⁾. In addition, Yokoyama *et al.* reported that Mg deficiency causes the increase in NO production⁹⁾. Therefore, an increase in NO production by the Mg deficiency may be caused by an increase in Ca²⁺ content in the lens, resulting in acceleration of cataract development. On the other hand, the onset of cataract development from cataractous SCR fed on a high Mg diet was similar to those from cataractous SCR fed on a standard Mg diet. These results suggest that an appropriate supplementation of Mg is important in preventing cataract development in SCR.

Further studies are needed to elucidate the precise mechanisms of acceleration of cataract development as a result of Mg deficiency. We are now in the progress of investigating iNOS protein expression and Ca²⁺ regulation in SCR fed on a low Mg diet. These findings provide signifi-

cant information that can be used to design further studies for preventing cataracts.

5 CONCLUSION

We demonstrated the effect of cataract development in the SCR as a result of supplementation of a powder diet containing various Mg contents. The low Mg diet accelerated the onset of cataract development, probably caused by the rise in Ca²⁺ content in the lens. On the other hand, excessive supplementation of Mg caused a decrease in the growth, and an onset of cataract development from SCR fed on a high Mg diet was similar to those from SCR fed on a standard Mg diet. Appropriate supplementation of Mg may be important in preventing cataract development.

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