Delay of Cataract Development in the Shumiya Cataract Rat by the Administration of Drinking Water Containing High Concentration of Magnesium Ion

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We discovered that the cataract development in the Shumiya cataract rat (SCR) can be prevented by the administration of deep-sea drinking water (DDW). A standard diet based on the American Institute of Nutrition guidelines (AIN-76) and DDW containing a high mineral concentration such as low, medium and high Mg2+ content (50, 200 and 1000 mg of Mg2+/l, respectively) were used in this study. SCRs were freely fed with combinations of the standard diet and purified water or DDW during 5–15 weeks of age. The opacities of SCR lenses were documented by anterior eye segment analysis system EAS-1000. The onset of opacification of cataractous SCR lenses administered a combination of standard diet and purified water started at 11 weeks of age, and mature cataracts had formed at 13 weeks of age. However, the supplementation of Mg2+ by administration with medium DDW showed the greatest effect of delay of cataract onset in SCR. In addition, even cataractous SCR lenses at 14 weeks of age showed differences in opacity level. The opacification and Ca2+ of the lenses in cataractous SCR administered medium DDW were lower than those administered purified water. In conclusion, the present study demonstrates that administration of DDW potently delays cataract development in SCR, and this may be caused by inhibiting the increase in Ca2+ levels in the lens.

Key words  deep-sea drinking water; cataract; magnesium; calcium; Shumiya cataract rat

Mineral ion, an essential element, plays a very important biochemical role in the body. It has been recognized that mineral ion imbalance is associated with several major diseases, such as diabetes mellitus, hypertension, cardiovascular and cerebrovascular diseases and heart infarction.1–7) In addition, Iwata and Kinoshita reported mineral ion imbalance in the lens causes cataract.8,9) Any alteration in the optical homogeneity of the lens or decrease in its transparency is known as a cataract,10,11) and numerous factors have been implicated in its etiology. These involve genetic factors, diabetes, smoking, nutrition, the cumulative effect of X-rays, ultraviolet irradiation, and alteration in both endocrine and enzymatic equilibrium.12–17) Cataractous lenses have been found to have an altered distribution of the intracellular ionic environment. The concentration of calcium ion (Ca2+) was increased in many cataractous lenses. There have been many reports on the relationship between Mg2+ and cataract. The Mg2+ in the lens increased in diabetic14) and ultraviolet-irradiated rats.18) On the other hand, lipid peroxides19) and cigarette smoke17) reduced the Mg2+ in the lens. In human senile cataract, Dilsiz et al. reported that lens ionic imbalance with increased levels of calcium and sodium, coupled with decreased levels of magnesium and potassium, is related to cataract development.20) These lens ionic imbalances arise as a result of changes to lens membrane characteristics causing an increase in lens membrane permeability.

Over the past several decades, there have been many studies exploring the mechanisms of cataract development.21) Currently, reactive oxygen species (ROS), induced by UV rays in sunlight, are considered to be important in perturbing lens homeostasis. Therefore, exposure to ROS results in a breakdown of lens homeostasis, and the Ca2+ concentration in the lens becomes elevated. The elevated Ca2+ concentration in the lens has been induced to activate calpain, a Ca2+-dependent protease. Furthermore, the degradation of lens proteins such as crystallin proteins would result in an opaque lens.22) This ROS is enhanced by hyperglycemia and Mg deficiency.23)

The Shumiya cataract rat (SCR), which was established by Shumiya and Nagase, is a hereditary cataractous rat strain.24) 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%.25) Previous investigations have revealed that oxidized glutathione concentrations in the SCR lens are increased, and reduced glutathione values are decreased.26) The proteolysis of some crystallins and cytoskeletal proteins was significantly enhanced in cataractous SCR lenses. The calcium concentrations in cataractous lenses rise markedly with age compared with non-cataractous lenses, and the autolytic product of calpain is also detected in cataractous lenses.27) It is noteworthy that SCR cataracts are not diabetic cataracts. Therefore, SCR should be a useful model for studies to reveal the mechanism of senile cataract development and the effect of nutrition.

In the present report, we investigated whether a balanced supplementation of mineral affects cataract development in SCR.

MATERIALS AND METHODS

Animals and Materials  The rats used were SCRs aged
5 to 15 weeks. They were housed under standard conditions (12 h/d fluorescent light (07:00—19:00), 25 °C room temperature) and fed with a combination of a standard diet based on American Institute of Nutrition guidelines (AIN-76) and various water obtained from AKO KASEI Co., Ltd. (Hyogo, Japan) such as purified water, deep-sea drinking water (DDW) and magnesium water. SCR strain rats were maintained in the Tokyo Metropolitan Institute of Gerontology (Tokyo, Japan). The standard 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. The compositions of the DDW and magnesium water are shown Table 1. Magnesium water including only magnesium (Mg2+; 200 mg/l) was prepared by MgSO4 and purified water. Lens opacity in SCR appears in exactly 2/3 of animals; the remainder has 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 age. Animal experiments were performed in accordance with the ARVO Resolution on the Use of Animals in Research. A Mg test kit and Ca test kit were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The parameters for the kinetics of Mg2+ and Ca2+ concentrations.

**Assay of the Mg2+ and Ca2+ Absorption Using in Situ Loop Technique** Male SCRs weighing 250—300 g and fasted overnight were anesthetized with pentobarbital (30 mg/kg). A small midline portion incision allowed the gentle exposure of a 4—5-cm target portion of intestine. This target small intestine was selected because of its suitable vascular supply to collect venous blood, and was washed gently with saline solution. An 8-cm length of silicon tubing (O.D: 2.0 mm, I.D: 1.0 mm, TERUMO Corp., Tokyo, Japan) was inserted in one end of the intestine and tied securely with a surgical suture. The opposite end was tied and 1.5—2.0 ml of medium DDW or standard diet suspended by purified water (Mg2+; 200 mg/l) was injected through the tube. Heparin (10 mg/kg) was injected in the femoral vein. The mesenteric vein was cannulated with an appropriate size of polyethylene tubing (Hibiki Co., Tokyo, Japan), and all venous blood was collected in a micro tube. This blood was centrifuged at 10000 rpm for 30 min at 4 °C, and the serum obtained was used for the determination of Mg2+ and Ca2+ concentrations. The Mg2+ concentration in the serum was measured by glutokinase enzymatic method using the Mg test kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and UV-2200 (Shimadzu Corp., Kyoto, Japan). The Ca2+ concentration in the serum was determined by the methyl xylenol blue colorimetric method using the Ca test kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and CL-770 (Shimadzu Corp., Kyoto, 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, in pixels, was analyzed by a computer using image analysis software connected to the EAS-1000 system. The total area of opacity of the lenses, expressed as pixels, was calculated by the following equation:

\[
\text{pixels within opacity (pixel)} = \text{pixels of cataractous SCR eye} - \text{pixels of non-cataractous SCR eyes}
\]

**Assay of Mg2+ and Ca2+ Contents in SCR Lens** Lenses taken from SCRs at 15 weeks of age were homogenized in phosphate-buffered saline (pH 7.4) on ice. The lens homogenates were centrifuged at 10000 rpm for 30 min at 4 °C, and the supernatant was used for measurements of Mg2+ and Ca2+ concentrations. The conditions of this experiment are described in “Assay of the Mg2+ and Ca2+ Absorption using in situ Loop Technique.” The Mg2+ and Ca2+ contents (μmol/g wet weight) in lenses were expressed as the ratio to the wet weight of lens.

**Statistical Analysis** The data are expressed as the mean±standard error of the mean. Statistical difference were performed using the unpaired Student’s or Aspin-Welch’s t-test and multiple groups were evaluated by one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison. p values of less than 0.05 were considered significant.

**RESULTS**

**Effect of Administration of Deep-Sea Drinking Waters on Serum Mg2+ and Ca2+ Concentration and Growth of SCRs** The medium DDW or standard diet suspended by purified water was used in the absorption experiment using in situ loop technique. The Mg2+ concentration of standard diet (1.53±0.01 μmol/ml, mean±S.E. of 3 samples) is lower than that of medium DDW (8.70±0.74 μmol/ml, mean±S.E. of 3 samples), and the solubility as ionization shows about 20%.

**Table 1. Mineral Composition of the Deep-Sea Drinking Water Used in This Experiment**

<table>
<thead>
<tr>
<th>Element</th>
<th>Low DDW (mg/l)</th>
<th>Medium DDW (mg/l)</th>
<th>High DDW (mg/l)</th>
<th>Magnesium water (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium</td>
<td>50</td>
<td>200</td>
<td>1000</td>
<td>200</td>
</tr>
<tr>
<td>Calcium</td>
<td>17.75</td>
<td>71</td>
<td>355</td>
<td>0</td>
</tr>
<tr>
<td>Sodium</td>
<td>18.5</td>
<td>74</td>
<td>370</td>
<td>0</td>
</tr>
<tr>
<td>Potassium</td>
<td>17.25</td>
<td>69</td>
<td>345</td>
<td>0</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.000013</td>
<td>0.000050</td>
<td>0.002500</td>
<td>0</td>
</tr>
<tr>
<td>Iron</td>
<td>0.0001</td>
<td>0.0004</td>
<td>0.0020</td>
<td>0</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.00225</td>
<td>0.0090</td>
<td>0.0450</td>
<td>0</td>
</tr>
<tr>
<td>Copper</td>
<td>0.001</td>
<td>0.004</td>
<td>0.020</td>
<td>0</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.001</td>
<td>0.004</td>
<td>0.020</td>
<td>0</td>
</tr>
<tr>
<td>Iodine</td>
<td>0.0023</td>
<td>0.0090</td>
<td>0.0450</td>
<td>0</td>
</tr>
<tr>
<td>Selenium</td>
<td>0.0003</td>
<td>0.0010</td>
<td>0.0050</td>
<td>0</td>
</tr>
</tbody>
</table>

**Image Analysis for Cataract Development in SCR** This was performed as described by Ito et al. The pupils of the SCRs were dilated by instillation of 0.1% pivalephine (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, in pixels, was analyzed by a computer using image analysis software connected to the EAS-1000 system. The total area of opacity of the lenses, expressed as pixels, was calculated by the following equation:

\[
\text{pixels within opacity (pixel)} = \text{pixels of cataractous SCR eye} - \text{pixels of non-cataractous SCR eyes}
\]

The lens image was analyzed by a computer using image analysis software connected to the EAS-1000 system. The total area of opacity of the lenses, expressed as pixels, was calculated by the following equation:

\[
C = kr + C_0
\]

where \( C \) (μg/ml) is the Mg2+ concentration at time \( t \) (min), \( C_0 \) (μg/ml) is the Mg2+ concentration at steady state (without administration of standard diet or medium DDW), and \( k \) (μg/ml/min) is the Mg2+ absorption rate constant.
Figure 1 shows Mg\(^{2+}\) absorption from the SCR small intestine into blood after injections of standard diet or medium DDW. After the injection of saline, the Mg\(^{2+}\) concentration maintained a steady value (approximately 1.00 \(\mu\)mol/ml). The Mg\(^{2+}\) concentration after the injection of standard diet increased (approximately 1.65 \(\mu\)mol/ml), reaching a plateau at 70 min. The Mg\(^{2+}\) concentration after the injection of medium DDW increased up to 90 min. The Mg\(^{2+}\) concentration after the injection of medium DDW was higher than that of standard diet. Table 2 shows the kinetic parameters for Mg\(^{2+}\) absorption in SCRs. The Mg\(^{2+}\) absorption rate constant \((k)\) and \(AUC_{0\rightarrow90}\) of medium DDW were significant higher than that of standard diet.

In addition, we measured Ca\(^{2+}\) absorption through the SCR small intestine into blood using the same samples. In contrast to the Mg\(^{2+}\) absorption, the serum Ca\(^{2+}\) concentration was maintained at constant (approximately 0.21 \(\mu\)mol/ml), and no significant difference between standard diet and medium DDW was seen.

The growth curve of SCRs administered DDW was almost same as that of SCRs administered purified water. The growth curves of the female SCRs also showed similar patterns to the male SCRs, but the body weights were approximately 70% in all male SCRs.

**Effect of Administration of Deep-Sea Drinking Waters on Cataract Developments in SCRs** Figure 2 depicts Scheimpflug slit images of eyes of cataractous SCR and the effects of administration of DDWs on these, as documented by EAS-1000. Figure 3 shows that the delay effects of cataract development in cataractous SCRs were administered various DDWs. During 6—10 weeks of age, the opacity of the lenses from cataractous SCR in this study was similar to

<table>
<thead>
<tr>
<th>Group</th>
<th>(k) ((\mu)g/ml/min)</th>
<th>(C_{ss}) ((\mu)g/ml)</th>
<th>(C_{90}) ((\mu)g/ml)</th>
<th>(AUC_{0\rightarrow90}) ((\mu)g/ml · min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard diet</td>
<td>0.193±0.046</td>
<td>21.84±0.68</td>
<td>40.95±3.98</td>
<td>2834±229</td>
</tr>
<tr>
<td>Medium DDW</td>
<td>0.355±0.020*</td>
<td>20.92±1.54</td>
<td>46.43±1.26</td>
<td>3393±44**</td>
</tr>
</tbody>
</table>

*Each value is presented as means±S.E. of 4—6 independent rats. \(\ast \) \(p<0.01\), vs. standard diet (Student’s \(t\)-test); \(\ast\ast \) \(p<0.05\), vs. standard diet (Welch’s \(t\)-test).*

**Fig. 1. Changes in Serum Mg\(^{2+}\) Concentration after Injection of Standard Diet or Medium DDW into SCR Small Intestine** Standard diet (●), Medium DDW (○), Saline (▲). The data are presented as means±S.E. of 4—6 independent rats.

**Fig. 2. Scheimpflug Slit Images of Lenses from Cataractous SCR Administered with or without DDWs** Scheimpflug slit images were obtained by an anterior eye segment analysis system (EAS-1000) from 10 to 14 weeks. (A) Purified water administered non-cataractous SCR lens, (B) purified water administered cataractous SCR lens, (C) low DDW administered cataractous SCR lens, (D) medium DDW administered cataractous SCR lens, (E) magnesium water administered cataractous SCR lens, (F) high DDW administered cataractous SCR lens. 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 Administered with or without DDWs** The cataractous SCR was administered various DDWs. Purified water administered cataractous SCR (●), low DDW administered cataractous SCR (◆), medium DDW administered cataractous SCR (▲), magnesium water administered cataractous SCR (●), high DDW administered 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±S.E. of 8—20 independent rat lenses. \(\ast \) \(p<0.05\), vs. purified water administered cataractous SCR (Dunnett’s multiple comparison).
that of non-cataractous SCR lenses. The opacification of lenses from cataractous SCR administered purified water started at 11 weeks of age, and mature cataracts had formed at 13 weeks. On the other hand, the lenses in cataractous SCR administered medium DDW were less opaque than those in cataractous SCR administered purified water. In addition, a similar phenomenon was observed in cataractous SCR administered magnesium water. However, the opacity of lens was not found to decrease with the increase in magnesium content in DDW. The opacity of the lenses from SCR administered high DDW with high magnesium content was similar to that in cataractous SCR administered purified water (Figs. 2, 3).

In the cataractous SCR (15 weeks of age), the opacity levels of lenses from cataractous SCR administered various diets showed a difference. The opacity levels of the lenses in cataractous SCR administered medium DDW or magnesium water were lower than those of cataractous SCR administered purified water. The cataractous SCR lenses administered medium DDW or magnesium water showed less opacity at 14 weeks of age, maintaining low 

**DISCUSSION**

Deep-sea water has a high mineral concentration such as Mg²⁺ and Ca²⁺ compared with surface and middle-sea water. DDW, which is prepared by desalinizing deep-sea water by 99.9%, contains high concentrations of Mg²⁺, which is prepared by desalinizing deep-sea water by 99.9%, contains high concentrations of Mg²⁺, and adjustments can be made to these ion concentrations. Drinking this DDW can be a balanced intake of minerals. Now, DDW is used as an Mg²⁺ supplement in Japan. In this report, we demonstrated a delaying effect of cataract development in SCR by the administration of DDW, which enables the effective intake of Mg²⁺.

First, we determined the absorption rate constant of Mg²⁺ from DDW using the in situ loop technique. The absorption rate constants of Mg²⁺ from DDW were higher than those of the suspension of standard diet (Fig. 1, Table 2). These results can be explained by the solubility as ionization in diet being lower than that in DDW. On the other hand, the absorption rate constants of Ca²⁺ between DDW and the suspension of standard diet showed no obvious differences.

Magnesium oxide is widely used as an antacid, laxative or magnesium supplement. Therefore, a decrease in the growth curves of SCRs administered DDW was observed compared with that of SCRs administered purified water. These results strongly suggest that DDW is a suitable form for supplying Mg²⁺ to the body.

This study showed that cataract development in SCRs was delayed by the administration of DDW (Fig. 3). These results suggest that the Mg²⁺ concentration in DDW is related to cataract development, since cataract development was significantly delayed in SCR administered magnesium water. In addition, opacity levels in cataractous SCR aged 14 weeks also decreased in cataractous SCR administered medium DDW or magnesium water. The change in opacity levels can be explained as the regulation of the change in Ca²⁺ in the lens, since even in lenses from cataractous SCR aged 15 weeks, the increase in Ca²⁺ concentration in lenses from cataractous SCR administered medium DDW or magnesium water were lower than those of cataractous SCR administered purified water (Table 3). On the other hand, opacity of lens was not found to decrease with the increase in magnesium content in DDW. Ribaya-Mercado and Gershoff also reported that galactose-induced cataract development was significantly accelerated in the high Mg-fed group. From these facts, it is possible that an appropriate supplementation of Mg²⁺ may cause delay of cataract development in SCR.

In the lens, the complex homeostasis of Ca²⁺ is regulated by various functions, such as Ca²⁺-ATPase, the membrane sulfhydryl group and mitochondria. Trevithick et al. reported that several types of mitochondrial damage lead to Ca²⁺ release and rat lens opacity. In addition, Nishikawa et al. reported that nitric oxide (NO) from inducible NO synthase (iNOS) decreased ATP levels and that the levels of cytosolic Ca²⁺ were significantly elevated by NO. This NO release was enhanced by Mg²⁺ insufficiency. We also showed that the high Ca²⁺ influx into lens cells resulted in cataract development in the SCR, and the induction of iNOS protein occurred prior to the elevation of Ca²⁺ content in the lens. Taking these findings together, it is conceivable that in the lenses of SCR, iNOS in first induced, leading to the production of NO, and then NO may cause the mitochondrial damage resulting in the Ca²⁺ influx into the lens. A moderate supplementation of Mg²⁺ levels may prevent NO production in the lens, suppressing Ca²⁺ influx into the lens.

Further studies are needed to elucidate the relationship between delay of cataract development and Mg²⁺ content. Therefore, we are now in the progress of investigating the Mg²⁺ effect in the human lens cells using a recently established human lens epithelial cell line, SRA01/04.

In summary, the present study demonstrated that an appro-

### Table 3. The Contents of Ca²⁺ and Mg²⁺ in the Crystalline Lens of Non-cataractous and Cataractous SCR at 15 Weeks of Age

<table>
<thead>
<tr>
<th>Drinking water</th>
<th>SCR</th>
<th>Ca²⁺ content (μmol/g wet weight)</th>
<th>Mg²⁺ content (μmol/g wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purified water</td>
<td>Non-cataractous</td>
<td>0.92±0.16*</td>
<td>32.0±0.2*</td>
</tr>
<tr>
<td>Purified water</td>
<td>Cataractous</td>
<td>2.53±0.08</td>
<td>25.3±1.2</td>
</tr>
<tr>
<td>Low DDW</td>
<td>Cataractous</td>
<td>2.33±0.25</td>
<td>26.6±0.9</td>
</tr>
<tr>
<td>Medium DDW</td>
<td>Cataractous</td>
<td>1.96±0.26**</td>
<td>27.4±0.8</td>
</tr>
<tr>
<td>Magnesium water</td>
<td>Cataractous</td>
<td>1.93±0.23**</td>
<td>26.7±1.4</td>
</tr>
<tr>
<td>High DDW</td>
<td>Cataractous</td>
<td>2.60±0.09</td>
<td>25.5±1.8</td>
</tr>
</tbody>
</table>

SCRs were fed with combination of standard diet and various water. The data are presented as means±S.E. of 4—8 independent rat lenses. *p<0.01, vs. purified water administered cataractous SCR; **p<0.05, vs. purified water administered cataractous SCR (Dunnett’s multiple comparison).
priate supplementation of Mg$^{2+}$ delays cataract development in SCR. This mechanism suggests that Mg$^{2+}$ suppresses Ca$^{2+}$ influx into the lens. The DDW prepared from deep-sea water is a suitable for supplying Mg$^{2+}$ to the body. These findings provide significant information that can be used to design further studies for preventing cataracts.

REFERENCES