Clinical significance of serum biochemistry changes in mice with targeted disruption of βB2–crystallin gene

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Abstract

AIM: To explore the pathogenesis, influencing factors, ways of medical intervention and evaluation indicators of cataract by observing changes in serum biochemical indices in mice with targeted disruption of β B2-crystallin.

METHODS: Nine 6-week-old male mice with targeted knockout of β B2-crystallin were used as the study group, and nine age- and sex-matched normal wild-type mice as the control group. The genotype of the modeled mice was identified by PCR technique. Tropicamide and phenylephrine eye drops were used as the cycloplegic agents to observe changes in lens opacity with a slit-lamp. The lens was then removed and blood was collected for biochemical evaluation in the serum.

RESULTS: Two genotypes were successfully identified by PCR technique. Slit-lamp observation showed that the lens cortex was opaque and GSH level in the lens cortex was remarkably decreased in mice with β B2-crystallin deficiency compared with the control group ($P<0.01)$. Serum Na+, Cl−, Ca2+, Mg2+ and Fe2+ levels, ALT and AST activities, and TP, ALP, Cr, TC, GLU content were decreased significantly compared with the control group ($P<0.05$). There was no difference in LDH, P, Cu2+, K+ levels between the two groups ($P>0.05$).

CONCLUSION: Compared with the wild-type mice, serum biochemical indices underwent significant changes in mice with targeted disruption of β B2-crystallin gene, especially with abnormal distribution of Na+&Ca2+, which induced the formation of cataract.

KEYWORDS: β B2-crystallin; knockout; cataract; mice; serum biochemistry
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INTRODUCTION

Crystallins are the major structural protein components of the vertebrate eye lens. They are built in the same manner and contain α-, β- and γ-crystallins according to migration in the electric field. α-crystallins are recognized to function both as structural proteins and as chaperones in the lens,[1,2] but little is known about β-crystallins. βB2-crystallin is significantly higher compared with others in the β-crystallin family, and its water-soluble components increase abnormally with age. The regular structure and water-soluble ingredient point to an important role in maintaining lens high refractive index and transparency.[3]

To understand further the function of βB2-crystallin gene and corresponding protein, we generated mice with targeted deletion of the βB2-crystallin gene,[4] and found that mice with βB2-crystallin deficiency were accompanied with marked lens opacity[5], but the mechanism has not been fully understood.

Previous studies showed that biochemical indices underwent obvious changes in cataract lenses, as represented by increased concentrations of Ca2+ and Na+, decreased K+ [6], and low level of GSH, which is regarded as an indicator of cataract. But experiments on serum biochemical features of cataract have rarely been reported. The objective of our experiment was to understand further the differences in serum biochemistry between mice with βB2-crystallin deficiency and normal wild-type mice, and explore the internal mechanism from the point of electrolyte balance and causation of radical damage.

MATERIALS AND METHODS

Materials Mice with targeted disruption of βB2-crystallin
Serum biochemistry changes in βB2-crystallin’ mice
geneweregeneratedbyinsertinganeoexpressioncassette
toreplacethefirstandthesecondexonsandpreventingthe
productionofafunctionaltranscriptfromthislocus [4].Both
normal and βB2-crystallin deficiency mice were of C57BL/C
genetic background, housed and maintained in the
Laboratory Animal Center of the Second Military Medical
University under a 12-h light/dark cycle. Food and water
were provided ad libitum, and animal care was given in
compliance with the National Institutes of Health and
institutional guidelines on the use of laboratory and
experimental animals. Nine 6-week-old male mice with
targeted knockout of βB2-crystallin gene were used as the
study group, and nine age- and sex-matched normal
wild-type mice were used as the control group.

Reagents and instrument Total tissue DNA was extracted
with the kit from Tiangen biochemistry Co., Ltd; PCR
amplification was done using Tx Hot Start Version kit of
TaKaRa Co., Ltd. The following primers were designed and
synthesized to detect the presence or absence of the
βB2-crystallin gene product. The first pair of primers was
used to detect the inserted neo cassette, it was forward
5’-TGCGAGGCCAGAGGCACTTGTGTAGC-3’ and
reverse 5’-CTGAATGCTGTGATTTCAGCGTCAC-3’. The
second pair of primers was used to detect the wild-type
mice, it was forward: 5’-GGTCACCAGAAGGAGGAA-3’
and reverse: 5’-GGAGAAACTTGTGGGCTAA-3’. Primers
weresynthetizedbyIvitrogenCo.,Ltd.TheInstruments
used included HITACHI 7600-120 automatic biochemical
analyzer (Japan); The reagents: Luo Co., Ltd (TP, Mg),
Zhangjiang Bio-Medical Co., Ltd of Shanghai Fudan
(LDH); Kehua Shanghai Co., Ltd (ALT, ALP, AST); Desai
Diagnostic Systems, Inc (Glu); Japan’s Hitachi (Na, K, Cl);
DENUO Co., Ltd (Cr, TC, Fe); Water company (Ca, P) and
Randox of Britain (Fe, Cu).

Methods
Detection of PCR products The intercepted mouse tail
end (0.2-0.5cm) was homogenised and placed in PBS.
Then the mice genomic DNA was extracted using Univer-
sal Genomic DNA Extraction kit of TaKaRa Co., Ltd. PCR
products were analyzed on 1% agarose gel and purified to
identify and quantify by spectrophotometry. PCR conditions
were as follows: Initial denaturation was done at 94℃ for 2
min, followed by 40 cycles of denaturation at 94℃ for 35 sec,
annealing at 58℃ for 35 sec, and extension at 72℃ for 45 sec.

Detection for Lens Transparancy Tropicamide and
phenylephrine were used as the cycloplegic agent, one drop
every three min, three times for each eye, and 30 min later
changes in lens opacity were observed with a slit-lamp.

Biochemical Tests About 0.8ml blood was collected from
the eye orbit of the mice after diethyl ether treatment, and
then centrifuged at 3000g for 15 minutes after 30-min
tranquilization at room temperature. Following the
manufacturer’s instructions of the reagent kit, serum was
separated for detection of TP (Biuret method), Cr (Jaffe
method), TC (CHOD-PAP method), Na⁺, Cl⁻ and K⁺ (ion
selection electrode method), Ca²⁺ (Orthocresolphthalein
method), P (Ammonium molybdate method), GLU
(O-toluidine method), the enzyme activities of ALP using
the modified Bowers and McComb method, AST and ALT
by the colorimetric method of Reitman and Frankel, LDH
by the Sigma colorimetric method [7-9]. The biochemical
parameters were measured using a standard autoanalyser
with veterinary software.

Statistical Analysis For quantitative analysis of the data
Mean ± standard deviation was computed. The significance
of differences between test and control groups was verified
by analysis of two sample t-test. Statistical significance
was considered when the P value was < 0.05.

RESULTS

PCR Analysis The DNA samples of βB2-crystallin-
deficiency mice and wild-type mice were amplified by PCR
assays, the knockout mice amplified only the first fragment
while the wild-type only the second fragment (Figure 1).

Determination of Opacity and GSH Level in the Lens
Slit-lamp survey showed apparent opacity in the cortex of
lens (Figure 2A) and the GSH levels in lens was found to be
remarkably decreased in βB2-crystallin-deficient mice as
compared to wild type mice (P < 0.01, Figure 2B).

Level of Serum Ions Serum Na⁺, Cl⁻, Ca²⁺, Mg²⁺, and Fe²⁺
levels in experiment group was significantly decreased as
compared to the controls while P, Cu²⁺, K⁺ ions did not show
any significant difference (P > 0.05, Table 1).

Levels of Enzymes and Proteins Serum ALT and AST
activity, and TP, ALP, Cr, TC and GLU content were lower
in the study group than those found in the control group (P <
0.05). There was no significant difference in LDH activity
between the two groups (P > 0.05, Table 1).

Figure 1 Fragments amplified in βB2-crystallin-deficiency mice and wild-type mice by PCR. The knockout mice were only purified in the first fragment (lane 1), while the wild-type mice were the second fragment (lane 4). (I and II represent the amplification products of the inserted neo gene and wild-type, respectively). The length of the amplified fragment was 760bp in both the cases.
Figure 2 Detection of opacity in the lens under slit-lamp and levels of GSH in lens  
A: The lens of the knockout mouse was apparently opaque (Right), while no significant change was observed in the lens of the wild-type mouse (Left); B: The GSH level in the lens of the knockout mice was lower than that in the wild type (**P<0.01).

Table 1  Biochemical indices of βB2-crystallin-deficient mice and wild-type mice

<table>
<thead>
<tr>
<th>Test item</th>
<th>Study group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP (g/L)</td>
<td>19.67±7.20</td>
<td>39.83±15.98*</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>5.33±3.01</td>
<td>16.00±5.62**</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>43.67±13.53</td>
<td>63.00±12.46*</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>133.33±51.25</td>
<td>215.17±57.43*</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>260.00±175.84</td>
<td>319.83±126.69</td>
</tr>
<tr>
<td>GLU (mmol/L)</td>
<td>3.10±0.76</td>
<td>5.02±1.28</td>
</tr>
<tr>
<td>Cr (umol/L)</td>
<td>2.67±1.63</td>
<td>6.00±2.61*</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>1.05±0.48</td>
<td>2.12±0.83*</td>
</tr>
<tr>
<td>Fe (umol/L)</td>
<td>20.01±8.99</td>
<td>50.89±16.09**</td>
</tr>
<tr>
<td>Ca (mmol/L)</td>
<td>0.42±0.25</td>
<td>1.28±0.72*</td>
</tr>
<tr>
<td>Mg (mmol/L)</td>
<td>0.36±0.16</td>
<td>0.71±0.33*</td>
</tr>
<tr>
<td>P (mmol/L)</td>
<td>1.79±0.61</td>
<td>2.62±0.80</td>
</tr>
<tr>
<td>Cu (umol/L)</td>
<td>6.57±3.07</td>
<td>11.25±4.84</td>
</tr>
<tr>
<td>Na (mmol/L)</td>
<td>118.67±22.15</td>
<td>148.00±19.73*</td>
</tr>
<tr>
<td>K+ (mmol/L)</td>
<td>4.07±1.06</td>
<td>4.87±1.16</td>
</tr>
<tr>
<td>Cl− (mmol/L)</td>
<td>85.67±15.46</td>
<td>104.17±11.97*</td>
</tr>
</tbody>
</table>

Comparison between experiment and control groups for different biochemical parameters estimated, *P<0.05, **P<0.01.

DISCUSSION

βB2-crystallin expression begins only after birth in rodents [9], almost all in the lens cortex. Studies [1] revealed that the water-solubility components of βB2-crystallin increased abnormally with increasing age, which points to an important role in maintaining the lens high refractive index and transparency [1]. Mouse cataract which was induced by knocking out βB2-crystallin gene shows cortical opacity [9] and the crystallins have higher oxidation susceptibility, lower thermal stability according to our previous study. The objective of this study was to explore the reason for cataract induced by Crybb2 deletion by observing changes in serum biochemical indices.

The mechanism of cataract formation is complex, including the explicit relationship between sugar and cataract, and the role of calcium and phosphorus. The abnormal level of serum total protein, albumin and liver function related enzyme can affect biochemical metabolism of the lens directly or indirectly, and induce cataract development. For example, decreased protein level in blood may lead to cataract formation by remote-effects lens nutritional [11]. In addition, ion balance is also an important functional factor for maintaining lens transparency. Studies have shown distribution of Ca2+ and Na+ is high and that of K+ was low in the cataract lens, possibly because calcium could influence cell membrane permeability and reduce Na+-K+ ATP enzyme activity. As a result, potassium and sodium ion channel open secondarily, which causes sodium retention and increases light scattering, eventually resulting in lens opacity. Dr Calivin [12] pointed out that calpain level became higher in the lens of experimental rats due to increased intracellular calcium. Other reports [13] demonstrated that low concentrations of calcium could also cause cataract by activating endopeptidase Calpain, lift Na+-K+ ATP enzyme activity and regulate intercellular link. In addition, taurine, as we all know, can lessen γ-crystallin leakage in the vitreous due to lens cell damage [14], and hence is considered a better anti-oxidant and anti-cataract drug. Moreover, taurine active transport is closely related to Na+-K+ ATP enzyme activity and regulate intercellular link. In addition, taurine active transport is closely related to Na+-K+ ATP enzyme activity and regulate intercellular link. In view of above assumption, we studied βB2-crystallin-deficient mice focusing on serum biochemistry index.

In this study our research show that the βB2-crystallin-deficiency mice exhibited clear opacity, and GSH level of lens was much lower than that in the Wild-type mice (Table 1), indicating the formation of cataract. Further the detection of serum biochemistry revealed that, the level of serum TP, ALP, ALT, AST in study group were lower than those in control group. Therefore, we speculated this abnormal event...
regulated biochemical metabolism, causing formation of cataract. The same result was observed in lenticular Ca²⁺, Na⁺ levels, which is contrary to the finding of other studies that Ca²⁺ and Na⁺ levels were increased in cataract lenses. The possible reason is that the physiological functions of organs and tissues in the model mice may be injured due to lack of βB2-crystallin. For example, liver damage affects ion transport resulting in calcium accumulation, calcium exchanging with blood \cite{15}. So biochemical function suffers severely, causing ion level disorder, and thereby inducing cataract gradually. Cruciani et al \cite{16} pointed out the linear relationship between Ca²⁺ and Na⁺ levels is related to cataractogenesis, which also explains our finding that cataract is induced by disproportion of Ca²⁺ and Na⁺. In addition, there was no significant difference in P, Cu²⁺ and K⁺ levels, which is consistent with the reports of Paterson CA in 1997 \cite{17}. But there is slight lowering of P, Cu²⁺ levels in experiment groups than in the normal ones, hence we deduce that synthesis of some enzymes will be cut back because P, Cu²⁺ are involved in important elements of most ion active transport enzyme, including some enzyme maintaining lens transparency. As a result, the enzyme can not effectively protect lens from oxidation or damage with aging. But the specific mechanism has not been identified. In short, we found that biochemical indices changed significantly in mice with targeted disruption of βB2 crystallin gene, probably due to the lack of βB2-crystallin, causing serum biochemical disorders and finally leading to cataractogenesis. Further study of βB2-crystallin function on related organization would be of significance in clarifying the pathogenesis of cataract and assisting the clinical diagnosis of cataract.

REFERENCES