·Basic Research·

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

Fen-Fen Xiang[1], Bei Cui[2], Qian Gao[1], Jian-Rong Zhang[1], Lin Liu[2], Wen-Jie Li[1]

[1]Department of Laboratory Diagnosis, Changhai Hospital, Second Military Medical University, Shanghai 200433, China
[2]Department of Ophthalmology, Changhai Hospital, Second Military Medical University, Shanghai 200433, China
Co-first author: Bei Cui
Correspondence to: Wen-Jie Li. Department of Laboratory Diagnosis, Changhai Hospital, Second Military Medical University, Shanghai 200433, China. wenjieli@pku.org.cn; Lin Liu. Department of Ophthalmology, Changhai Hospital, Second Military Medical University, Shanghai 200433, China. linliu@shl63.com
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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 genetype 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^-, Ca^{2+}, Mg^{2+} and Fe^{2+} 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, Cu^{2+}, 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^+&Ca^{2+}, 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 Ca^{2+} 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

gene were generated by inserting a neo expression cassette to replace the first and the second exons and preventing the production of a functional transcript from this locus [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'-TGCGAGGCCAGAGGC CACTTGTGTAGC-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'-GGAGAAACTTGTGGGCTA A-3'. Primers were synthetized by Ivitrogen Co., Ltd. The Instruments 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 Transpirancy 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 tranquillization 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), Ca^{2+} (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.

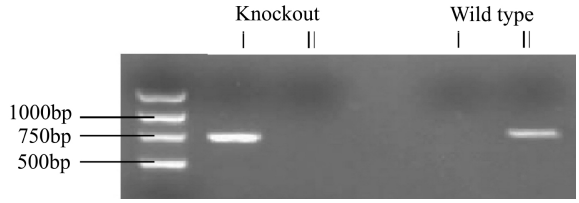

Figure 1 Fragments amplified in βB2 –crystallin –deficiency mice and wild –type mice by PCR The knockout mice were only purified in the first fragment (lane 1), while the wild-type mice were the second fragment (lane 4). (I and II represent the amplification products of the inserted neo gene and wild-type, respectively). The length of the amplified fragment was 760bp in both the cases.

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^-, Ca^{2+}, Mg^{2+}, and Fe^{2+} levels in experiment group was significantly decreased as compared to the controls while P, Cu^{2+}, 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).

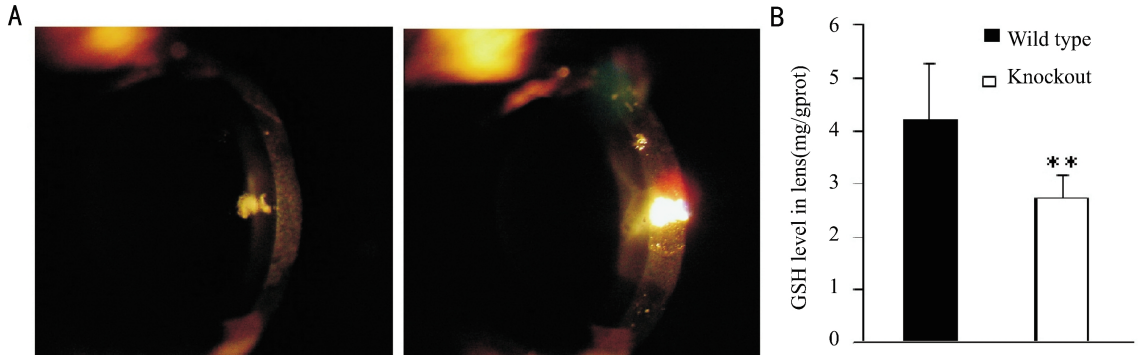

Figure 2 Detection of opacity in the lens under slit-lamp and levels of GSH in lens A: The lens of the knockout mice was apparently opaque (Right), while no significant change was observed in the lens of the wild-type mouse (Left); B: The GSH level in the lens of the knockout mice was lower than that was in the wild type (** P<0.01).

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

Test item	Study group	Control group
TP(g/L)	19.67±7.20	39.83±15.98*
ALT(U/L)	5.33±3.01	16.00±5.62**
AST(U/L)	43.67±13.53	63.00±12.46*
ALP(U/L)	133.33±51.25	215.17±57.43*
LDH(U/L)	260.00±175.84	319.83±126.69
GLU(mmol/L)	3.10±0.76	5.02±1.28
Cr(umol/L)	2.67±1.63	6.00±2.61*
TC(mmol/L)	1.05±0.48	2.12±0.83*
Fe^{2+}(umol/L)	20.01±8.99	50.89±16.09**
Ca^{2+}(mmol/L)	0.42±0.25	1.28±0.72*
Mg^{2+}(mmol/L)	0.36±0.16	0.71±0.33*
P(mmol/L)	1.79±0.61	2.62±0.80
Cu^{2+}(umol/L)	6.57±3.07	11.25±4.84
Na^{+}(mmol/L)	118.67±22.15	148.00±19.73*
K^{+}(mmol/L)	4.07±1.06	4.87±1.16
Cl^{-}(mmol/L)	85.67±15.46	104.17±11.97*

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 [10], almost all in the lens cortex. Studies [3] 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 [3]. Mouse cataract which was induced by knocking out βB2-crystallin gene shows cortical opacity [5] 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 tutritional [11]. In addition, ion balance is also important functional factor for maintaining lens transparency. Studies have shown distribution of Ca^{2+} and Na^{+} was 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, which we speculate will alterate the sodium, potassium in serum, affect taurine active transport and influence the occurrence of cataract. 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 biochemisty 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 Ca^{2+}, Na^{+} levels, which is contrary to the finding of other studies that Ca^{2+} 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 [15]. So biochemical function suffers severely, causing ion level disorder, and thereby inducing cataract gradually. Cruciania *et al* [16] pointed out the linear relationship between Ca^{2+} and Na^{+} levels is related to cataractogenesis, which also explains our finding that cataract is induced by disproportion of Ca^{2+} and Na^{+}. In addition, there was no significant difference in P, Cu^{2+} and K^{+} levels, which is consistent with the reports of Paterson CA in 1997 [17]. But there is slight lowering of P, Cu^{2+} levels in experiment groups than in the normal ones, hence we deduce that synthesis of some enzymes will be cut back because P, Cu^{2+} 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.

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