Indian J Med Res 150, October 2019, pp 407-411
DOI: 10.4103/ijmr.IJMR_50_18

Quick Response Code:

Anticataractogenic effect of betaine in chick embryo hydrocortisone-induced cataract model

Reşat Duman[1], Tolga Ertekin[2], Rahmi Duman[1], Ayhan Vurmaz[3], Ersan Çetinkaya[4] & Hilal Güzel[2]

Departments of [1]Ophthalmology, [2]Anatomy & [3]Clinical Biochemistry, School of Medicine, Afyon Kocatepe University, Afyonkarahisar & [4]Department of Ophthalmology, Antalya Education & Research Hospital, Antalya, Turkey

Received January 6, 2018

***Background & objectives*: Cataract is one of the leading causes of blindness in the world. The aim of the present study was to investigate anticataractogenic effect of betaine in chick embryo hydrocortisone (HC)-induced cataract model.**

***Methods*: The study included 60 fertilized eggs divided into six groups each having 10 eggs: one group treated with only HC (HC group); three treated with both HC and different doses of betaine (HC/B 1.00, HC/B 0.50 and HC/B 0.25 groups) and two non-HC groups treated with only phosphate-buffered saline (PBS group) or betaine (B group). After the injections, lenses of the embryos were removed and classified into five stages according to the lens opacification. The amounts of reduced glutathione (GSH) in the removed lenses were measured.**

***Results*: All the lenses in non-HC-treated groups were clear, whereas in the HC-treated group, 90 per cent of the lenses had cataract (stages 4 and 5). The mean score of lens opacity was significantly lower in all HC/B groups compared to HC group (2.4-3.5 vs. 4.4, $P<0.05$). Among HC/B groups, the HC/B 0.25 group had significantly lower mean score of lens opacity compared to remaining HC/B groups treated with higher doses of betaine. In addition, the mean reduced GSH level was significantly higher in HC/B 0.25 group compared to HC, HC/B 1.00 and HC/B 0.50 groups ($P<0.001$).**

***Interpretation & conclusions*: The present results show beneficial anti-cataract and anti-oxidant effects of 0.25 µmol/egg betaine on HC-induced cataract in the chick embryo.**

Key words Betain - cataract - chick embryo - hydrocortisone - oxidative stress

It has been long known that prolonged glucocorticoid therapy leads to some ocular complications such as posterior subcapsular cataract[1]. Many experimental studies have been conducted on steroid-induced cataract (SIC) with various animal models such as rat, mouse, rabbit and chick embryo SIC models[1-5]. Although the exact molecular events causing SIC are not known, there have been many theories about aetiopathogenesis of SIC such as metabolic changes, osmotic failure, gene transcription events and oxidative stress in lens epithelial cells[6,7].

Betaine (N,N,N-trimethylglycine) is an organic osmolyte found in humans with important physiological functions such as assisting cell volume regulation, providing methyl group for the remethylation of homocysteine to methionine and taking a role in cellular antioxidant defence[8-10]. Previously, positive effects of betaine on many diseases such as diabetes, obesity, cancer and autism have been reported[11-14]. It is hypothesized that betaine, as an antioxidant, may prevent the onset of oxidative stress in the lens leading to cataract formation. For this purpose, in the present study, preventive effects of betaine were investigated on cataract formation using chick embryo SIC model.

Material & Methods

This study was carried out for one year from June to November 2017 in the department of Anatomy of Afyon Kocatepe University, Afyonkarahisar, Turkey. Sixty fertilized specific pathogen-free eggs were included in this experimental study. The study protocol was approved by the Ethics Committee for Animal Experiments of the University.

In this study, chick embryo SIC model was used which was first reported by Lee *et al*[15]. Several studies have shown that the anatomy and histology of the chick embryo lens tissues are similar in size and appearance to human lens tissues[15-17].

All eggs were placed in the incubator and monitored at 37.5°C and 65 per cent relative humidity. The eggs were randomly divided into six groups each having 10 eggs: one group treated with only hydrocortisone (0.5 µmol/0.1 ml) (HC group); three groups treated with both HC and different doses of betaine (both procured from Sigma-Aldrich, USA, HC/B 1.00, HC/B 0.50 and HC/B 0.25 groups) and two non-HC groups treated with only phosphate-buffered saline, *p*H 7.4 (PBS group) or betaine (B group). Betaine was dissolved in PBS at the following concentrations: 1 µmol /0.1 ml in group HC/B 1.00; 0.5 µmol/0.1 ml in group HC/B 0.50 and 0.25 µmol/0.1 ml in groups B and HC/B 0.25. Injections were given on day 15 of incubation and into the air sac (AS) of the eggs. In HC/B groups, 0.1 ml of betaine solution was injected to the AS 3 h after the injection of HC. After injection, the puncture was sealed with sterile cellophane tape, and the eggs were further incubated for 48 h in the incubator.

Evaluation of opacity of removed lenses: On day 17 of incubation (48 h after the injection), the lenses were removed from chick embryos under the dissecting microscope (Carl Zeiss, OMPI Pico, Germany) with corneal limbus incision. The states of the lenses were determined under a stereoscopic microscope (Zeiss, Stemi 2000-C, Germany), and their photographs were taken. Two lenses of each chick embryo served as one sample.

A previously described staging system[15] was used to score the lenses on a five-grade scale: (*i*) clear lens (no lens opacity); (*ii*) lens with a faint opaque ring between the cortical region and the nuclear region; (*iii*) lens with a distinct opaque ring between these regions; (*iv*) lens with a pinhole-sized clear area in an opaque nucleus; and (*v*) lens with an opaque nucleus.

Measurement of reduced glutathione (*GSH*) *levels in the removed lenses*: The removed lenses were immediately frozen and stored at −80°C until the measurement of the reduced glutathione (GSH) level. The sample was sonicated in ice with 0.1 M (*p*H 7.4) phosphate buffer. The homogenates were centrifuged at 10,000×*g* for 15 min. GSH levels were determined by Glutathione Assay Kit (Chromsystems Diagnostics; Munich/Germany) with high-performance liquid chromatography (HPLC) method using Ultimate 3000 HPLC device with a HPLC fluorescence detector (Ex: 385, Em: 515 nm) supplied by ThermoFisher Scientific, USA.

Statistical analysis: The statistical analysis was performed with SPSS software v18.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were presented as a mean±standard deviation and categorical variables as frequencies and percentages. Differences between groups were determined using one-way analysis of variance test.

Results

On day 17 after injection, it was observed that three eggs in group HC/B 1.00 and two in group HC/B 0.50 did not develop; so, these eggs were excluded from the study. Development of the remaining eggs in these groups was not compatible with that of day 17 showing development retardation in these groups.

Lens evaluation in all groups on day 17 showed that all the lenses (100%) were clear (stage 1) in groups only injected with PBS or betain, whereas cataractous lenses of stages 4 and 5 were seen in 90 per cent of the cases in HC group (Figure). In HC/B groups, the lens opacity score more than stage 3 was seen in 57.1 per

Table. Opacity scores of removed lenses and mean reduced glutathione levels in study groups

Group (sn)	Opacity scores of removed lenses†						GSH levels (µmol/l), mean±SD
	1	2	3	4	5	Mean (minimum-maximum)	
HC/B 1.00 (7)	1	1	1	1	3	3.5 (1-5)	45.4±13.9
HC/B 0.50 (8)	1	1	2	1	3	3.5 (1-5)	106.3±48
HC/B 0.25 (10)	2	3	4	1	0	2.4 (1-4)	424.3±132
HC (10)	0	0	1	4	5	4.4 (3-5)	229.1±71
PBS (10)	10	0	0	0	0	1	294.9±62
B (10)	10	0	0	0	0	1	523.3±219

In each group, two lenses of each chick embryo served as one sample; †Numbers show the numbers of lens samples with each opacity score. HC, hydrocortisone succinate sodium; PBS, phosphate-buffered saline; B, betaine; GSH, reduced glutathione; sn, sample number; SD, standard deviation

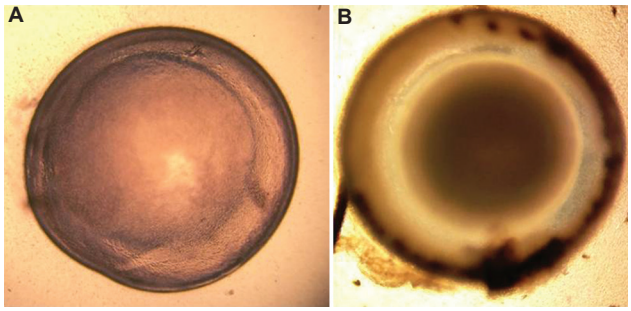

Figure. (**A**) Lens image with grade 1 cataract in the group treated with betaine, (**B**) lens image with grade 5 cataract in the group treated with hydrocortisone.

cent (HC/B 1.00), 50 per cent (HC/B 0.50) and 10 per cent (HC/B 0.25) of the removed lenses, respectively. The mean score of lens opacity was significantly lower in all HC/B groups compared to HC group (2.4-3.5 vs. 4.4, $P<0.05$). In addition, the mean score of lens opacity was significantly lower in HC/B 0.25 group compared to HC/B 1.00 and HC/B 0.50 groups, $P<0.05$). However, the mean scores of lens opacity did not significantly differ between HC/B 1.00 and HC/B 0.50 groups.

The mean GSH level in lenses was significantly higher in HC/B 0.25 group compared to HC, HC/B 1.00 and HC/B 0.50 groups ($P<0.001$). Significantly lower mean GSH levels in HC/B 0.50 and HC/B 1.00 groups compared to remaining groups were in consistence with observed developmental retardation of the eggs in these groups. Mean lens opacity scores and GSH levels within each study group are shown in the Table.

Betaine treatment with 0.25 µmol/egg prevented cataract formation without any developmental problems in chick embryos, whereas treatment with higher dosages (0.50/1.00 µmol/egg) of betaine caused developmental retardation with lower GSH levels.

Discussion

The present study was aimed to examine the preventive effects of different doses of betaine on cataract formation using *in vivo* chick embryo SIC model. Chick embryo SIC model has been used as an efficient animal cataract model to study the anti-cataract potential of several agents[16-20].

Betaine is one of the several organic osmolytes involved in cellular antioxidant defence, protein stabilization and stress responses. Moreover, the level of betaine is known as a dominating osmolyte in placenta and renal medulla tissue[21]. The lenticular role of betaine is not exactly known[22]. The exact metabolic changes and repair processes occurring in the lens cells after an injury are not known. However, it has been shown that some osmolytes, especially methylamines like betaine, may have stabilizing effects on macromolecules[23]. This effect is of crucial importance to the lens fibre cells which have limited capacity of damage repair[24]. Some previous studies evaluating metabolic changes in the lens tissue after ultraviolet radiation (UVR)-induced damage have shown that absorbed UVR photons excite lens molecules and create free radicals which increase the oxidative stress on the lens[24,25]. In addition, studies evaluating metabolic profile of the lens tissue under normal and cataractous conditions using nuclear magnetic resonance spectroscopy have shown a significant decrease in the concentrations of lactate, succinate, taurine, betaine and myoinositol after UVB irradiation[26,27]. A significant decrease in osmolytes such as taurine, myoinositol and betaine has been reported to indicate a loss of homeostasis and osmotic stress[26,27].

The anti-cataract effects of betaine shown in our study may be explained by functions of betaine in cellular osmotic homeostasis and anti-oxidant defence in lens fibre cells. The present study findings also showed that anti-cataract effect of betain was associated with lenticular GSH levels, suggesting that the betaine might suppress lens opacification *via* anti-oxidant functions.

One of the major findings of the present study was that betaine showed anti-cataract effect when given in optimum dosage. Treatment with 0.25 µmol/egg betain prevented cataract formation without any developmental problems in chick embryos, whereas treatment with higher dosages (0.50/1.00 µmol/egg) of betaine caused developmental retardation with lower GSH levels. It is known that overexpression of anti-oxidant systems leads to excess reducing capacity that can deplete reactive oxidative species, driving the cells to reductive stress. Furthermore, it has been shown that chronic consumption of anti-oxidant supplements may have pro-oxidant effects that can alter the cellular redox balance[28]. Betaine is an important human nutrient obtained from a variety of foods including wheat, shellfish, spinach and sugar beets[29,30]. Small sample size and the technical difficulties with the small chick embryo eyes were the major limitations of our study.

In conclusion, administration of a dose of 0.25 µmol/egg betaine showed a significant anti-cataract effect in chick embryo SIC model. This finding is significant as it shows betaine as a potential anti-oxidant agent preventing cataract formation.

***Financial support & sponsorship*:** None.

***Conflicts of Interest*:** None.

References

1. Black RL, Oglesby RB, Von Sallmann L, Bunım JJ. Posterior subcapsular cataracts induced by corticosteroids in patients with rheumatoid arthritis. *JAMA* 1960; *174* : 166-71.
2. Nishigori H, Lee JW, Iwatsuru M. An animal model for cataract research: Cataract formation in developing chick embryo by glucocorticoid. *Exp Eye Res* 1983; *36* : 617-21.
3. Shui YB, Vrensen GF, Kojima M. Experimentally induced steroid cataract in the rat: A scanning electron microscopic study. *Surv Ophthalmol* 1997; *42* (Suppl 1) : S127-32.
4. Rogoyski A, Trzcinska-Dabrowska Z. Corticosteroid-induced cataract and palatoschisis in the mouse fetus. *Am J Ophthalmol* 1969; *68* : 128-33.
5. Wallentin N, Lundberg C. Steroid and anti-CD18 treatment have no effect on after-cataract formation following surgery in rabbits. *Curr Eye Res* 2000; *20* : 384-93.
6. James ER. The etiology of steroid cataract. *J Ocul Pharmacol Ther* 2007; *23* : 403-20.
7. Jobling AI, Augusteyn RC. What causes steroid cataracts? A review of steroid-induced posterior subcapsular cataracts. *Clin Exp Optom* 2002; *85* : 61-75.
8. Schliess F, Häussinger D. The cellular hydration state: A critical determinant for cell death and survival. *Biol Chem* 2002; *383* : 577-83.
9. Lang F. Mechanisms and significance of cell volume regulation. *J Am Coll Nutr* 2007; *26* : 613S-23S.
10. Lever M, Slow S. The clinical significance of betaine, an osmolyte with a key role in methyl group metabolism. *Clin Biochem* 2010; *43* : 732-44.
11. Xu X, Gammon MD, Zeisel SH, Bradshaw PT, Wetmur JG, Teitelbaum SL, *et al.* High intakes of choline and betaine reduce breast cancer mortality in a population-based study. *FASEB J* 2009; *23* : 4022-8.
12. Dellow WJ, Chambers ST, Lever M, Lunt H, Robson RA. Elevated glycine betaine excretion in diabetes mellitus patients is associated with proximal tubular dysfunction and hyperglycemia. *Diabetes Res Clin Pract* 1999; *43* : 91-9.
13. Konstantinova SV, Tell GS, Vollset SE, Nygård O, Bleie Ø, Ueland PM. Divergent associations of plasma choline and betaine with components of metabolic syndrome in middle age and elderly men and women. *J Nutr* 2008; *138* : 914-20.
14. James SJ, Cutler P, Melnyk S, Jernigan S, Janak L, Gaylor DW, *et al.* Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism. *Am J Clin Nutr* 2004; *80* : 1611-7.
15. Lee JW, Iwatsuru M, Nishigori H. Glucocorticoid-induced cataract of developing chick embryo as a screening model for anticataract agents. *J Ocul Pharmacol Ther* 1995; *11* : 533-41.
16. Velpandian T, Nirmal J, Gupta P, Vijayakumar AR, Ghose S. Evaluation of calcium dobesilate for its anti-cataract potential in experimental animal models. *Methods Find Exp Clin Pharmacol* 2010; *32* : 171-9.
17. Nishigori H, Lee JW, Iwatsuru M. Glucocorticoid-induced cataract of the developing chick embryo-prevention by propylene glycol. *Ophthalmic Res* 1995; *27* : 350-5.
18. Ishikawa S, Hashizume K, Nishigori H, Tezuka Y, Sanbe A, Kurosaka D, *et al.* Effect of astaxanthin on cataract formation induced by glucocorticoids in the chick embryo. *Curr Eye Res* 2015; *40* : 535-40.
19. Kosano H, Watanabe H, Nishigori H. Suppressive effects of thyroxine on glucocorticoid (gc)-induced metabolic changes and cataract formation on developing chick embryos. *Exp Eye Res* 2001; *72* : 643-8.
20. Nishigori H, Kosano H, Umeda IO, Nishigori H. Inhibition of glucocorticoid-induced cataracts in chick embryos by RU486: A model for studies on the role of glucocorticoids in development. *Life Sci* 2004; *75* : 3027-33.
21. Miller TJ, Hanson RD, Yancey PH. Developmental changes in organic osmolytes in prenatal and postnatal rat tissues. *Comp Biochem Physiol A Mol Integr Physiol* 2000; *125* : 45-56.
22. Rao PV, Garrow TA, John F, Garland D, Millian NS, Zigler JS Jr. Betaine-homocysteine methyltransferase is

a developmentally regulated enzyme crystallin in rhesus monkey lens. *J Biol Chem* 1998; *273* : 30669-74.

23. Yancey PH, Clark ME, Hand SC, Bowlus RD, Somero GN. Living with water stress: Evolution of osmolyte systems. *Science* 1982; *217* : 1214-22.
24. Spector A. Oxidative stress-induced cataract: Mechanism of action. *FASEB J* 1995; *9* : 1173-82.
25. Rose RC, Richer SP, Bode AM. Ocular oxidants and antioxidant protection. *Proc Soc Exp Biol Med* 1998; *217* : 397-407.
26. Risa Ø, Saether O, Löfgren S, Söderberg PG, Krane J, Midelfart A. Metabolic changes in rat lens after *in vivo* exposure to ultraviolet irradiation: Measurements by high resolution MAS 1H NMR spectroscopy. *Invest Ophthalmol Vis Sci* 2004; *45* : 1916-21.
27. Risa O, Saether O, Kakar M, Mody V, Löfgren S, Söderberg PG, *et al.* Time dependency of metabolic changes in rat lens after *in vivo* UVB irradiation analysed by HR-MAS 1H NMR spectroscopy. *Exp Eye Res* 2005; *81* : 407-14.
28. Pérez-Torres I, Guarner-Lans V, Rubio-Ruiz ME. Reductive stress in inflammation-associated diseases and the pro-oxidant effect of antioxidant agents. *Int J Mol Sci* 2017; *18*. pii: E2098.
29. Pryor JL, Craig SA, Swensen T. Effect of betaine supplementation on cycling sprint performance. *J Int Soc Sports Nutr* 2012; *9* : 12.
30. Cholewa JM, Hudson A, Cicholski T, Cervenka A, Barreno K, Broom K, *et al.* The effects of chronic betaine supplementation on body composition and performance in collegiate females: A double-blind, randomized, placebo controlled trial. *J Int Soc Sports Nutr* 2018; *15* : 37.

For correspondence: Dr Reşat Duman, Department of Ophthalmology, School of Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey
e-mail: resatduman@gmail.com