JIMA Vol. 69, No. 5 (Dec. 2017) pp. 265-272.

Original

Effect of green tea polyphenols on glucocorticoid-induced cataract formation in chick embryo

Kanako MAEDA [1], Kouhei HASHIZUME [1], Sayano ISHIKAWA [1], Satoshi OGASAWARA [1], Takashi OKUNO [1], Taro SEINO [1], Hideo NISHIGORI [2], Atsushi SANBE [2] and Daijiro KUROSAKA [1]

[1] Department of Ophthalmology, School of Medicine, Iwate Medical University, Morioka, Japan

[2] Department of Pharmacotherapeutics, School of Pharmacy, Iwate Medical University, Yahaba, Japan

(Received on January 20, 2017 & Accepted on February 16, 2017)

Abstract

The purpose of this research was to determine whether green tea polyphenols (GTP) prevent glucocorticoid-induced cataract formation in a chick embryo model.

Hydrocortisone hemisuccinate sodium (HC) (0.5 μmol/egg) was administered directly into the air chamber of the egg shells of chick embryos on day 15. The eggs were kept in an incubator under similar conditions and administered 200 μL GTP (1.00 mg/mL, HC+GTP group), or saline (HC-alone group) 3, 10, and 20 h after HC administration. After 48 h treatment, the lenses were removed from embryos and classified into five stages according to the developed opacity: I, no lens opacity; II or III, faint or clear white ring in the periphery of the lens nucleus, respectively; and IV or V , opacity of the lens nucleus not spreading or spreading to the center of the nucleus, respectively. The reduced glutathione (GSH) levels of the lenses were measured.

Opacity of score IV or higher was seen in 75% of lenses from the HC-alone group, and 0 % of the lenses from the HC + GTP group ($p < 0.001$, Chi-squared test), while the lens GSH levels were 5.41 ± 0.46 and 7.10 ±0.88 nmol/lens, respectively ($p < 0.001$, Sheffe's test). Therefore, GTP decreased the lens opacity and recovered the decreased glutathione level.

These findings suggest that the antioxidative effects of green tea polyphenols protect against glucocorticoid-induced cataract in chick embryos.

Key words : *cataract, glucocorticoid, green tea polyphenols, oxidative stress, chick embryo*

I. Introduction

Cataract refers to opacity of the lens associated with reduced visual acuity, which can be caused by various factors such as aging, diabetes mellitus, use of steroids, and trauma. While patients with cataract in developed countries have access to surgical treatment that can improve visual acuity, those in developing countries may not, and their condition often progresses to blindness. Thus, cataract remains the leading cause of blindness worldwide [1].

Recent epidemiological studies revealed that

Corresponding author: Kanako Maeda
s.kanako.0327@gmail.com

exposure to ultraviolet rays [2, 3], smoking [4-9], and insufficient vitamin C/E intake [10, 11] are risk factors for developing cataract. In addition, oxidative stress is known to be involved in the pathogenesis of cataract. Oxidative stress develops when the production of reactive oxygen species (ROS) overwhelms antioxidant defenses [12], and therefore antioxidants are thought to prevent cataract development by scavenging ROS, thereby decreasing oxidative stress.

Green tea has high levels of polyphenols (25%-35% of the dry base). In vivo and in vitro experiments have shown that green tea polyphenols (GTP) have a potent antioxidative activity [13-15] that may be useful in suppressing cataract development.

Therefore, in the present study, we evaluated the effects of GTP on the development of cataract in a chick embryo model of steroid-induced cataract.

II. Materials and Methods

1. Establishment of chick embryo model of steroid-induced cataract

Chick embryos were treated in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research. Based on a method described previously [16], fertilized hen eggs were incubated at a temperature and relative humidity of 37 ℃ and 68%, respectively. On day 15 (day 1, incubation day), hydrocortisone hemisuccinate sodium (HC) (Sigma-Aldrich, St. Louis, MO, USA) was injected at a dose of 0.5 μ mol/egg into the air chamber of each fertilized chicken egg to establish the chick embryo model of steroid-induced cataract.

2. Administration of GTP

GTP was purchased from Wako Pure Chemical Industries (Osaka, Japan) and was dissolved in saline to a concentration of 1.00 mg/mL. The GTP solution was added to the fertilized chicken eggs at a volume of 200 μL 3, 10, and 20 h after the injection of HC (HC + GTP group, n=19). In addition, non-HC (treated with 200 μL saline without HC, n =20) and HC-alone (treated with 0.5 μmol HC and 200 μL saline, n= 20) groups were established.

3. Evaluation of cataract

The necropsy examination of each embryo was carried out 48 h after the HC treatment. The chick embryo was removed from the egg, the corneal limbus was incised, and the lens was removed. The removed lens was subsequently examined macroscopically and the severety of opacity was scored using the following 5-grade scale: I, no lens opacity; II and III, faint and clear white ring in the lens nuclear periphery, respectively; and IV and V, opacity of the lens nucleus not spreading and spreading to the center of the nucleus, respectively, as described previously [17].

4. Measurement of reduced glutathione levels in the lens

The excised lenses were immediately frozen at −80℃ and stored at this temperature until the reduced glutathione (GSH) level was measured. Two lenses from each chick embryo served as one sample. The sample was removed from the deep freezer, placed in cool distilled water (0.4 mL), and then ultrasonically crushed in ice. The crushed sample was immediately mixed with 0.1 mL 20% cooled trichloroacetic acid, and centrifuged at 10,000 rpm for 5 min. The supernatant (100 μL) was harvested, and then placed in a 96-well

Table 1. Incidence of cataract in HC-treated developing chick embryos after GTP administration

	Stage of lenses at 48 hours after HC treatment						
	I	II	III	IV	V	p (vs. non-HC)	p (vs. HC-alone)
non-HC (n = 20)	20 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)		
HC-alone (n = 20)	2 (10%)	1 (5%)	2 (10%)	9 (45%)	6 (30%)	< 0.001	< 0.001
HC+GTP (n = 19)	13 (68%)	4 (21%)	2 (11%)	0 (0%)	0 (0%)	0.063	< 0.001

No opacity was seen in any of the lenses from the non-HC group. Opacity of score 4 or higher was seen in 15 (75.0 %) of the 20 lenses from the HC-alone group, 0 (0.0%) of the 19 lenses from the GTP treatment group.

microplate. Tris buffer (1M, pH 8, 95 μ L) and 5 mM 5,5'-diobis (2-nitrobenzoic acid) dissolved in methanol (5 μ L) were added to each well, followed by a 15-min incubation at room temperature after which the absorbance was measured at 415 nm. The microplate was similarly treated with cysteine (1, 2, 5, and 10 nmol/well), and the data obtained was used to construct a calibration curve.

5. Measurement of blood glucose level

Blood was sampled from each embryo 48h after the HC treatment, and the glucose level was measured using a Breeze 2 glucometer (Bayer, IN, USA).

6. Statistical analysis

The chi-squared test was used to compare the lens opacity. A one-way analysis of variance (ANOVA) and Sheffe's test as the post-hoc tests were used to compare the GSH levels of the lens and blood glucose levels. Differences with $p<0.05$ were regarded as statistically significant. The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 24 (SPSS Inc., Chicago, IL, USA).

III. Results

1. Lens opacity

Lens opacity was noted in the HC-treated group but not in the non-HC and GTP groups. Furthermore, lens opacity scores greater than III were reported for 85% and 11% of eggs in the HC-alone and HC+GTP groups,respectively. The lens opacity score 48 h after the HC treatment was significantly lower in the HC + GTP group than it was in the HC-alone group ($p < 0.001$, Table 1). Thus, GTP suppressed the HC-induced cataract formation.

2. GSH levels of the lenses

The GSH levels of the lens were 5.4 ± 0.5 nmol/lens in the HC-alone group, which was significantly lower than that in the non-HC group (9.2 ± 0.6 nmol/lens, $p < 0.001$). The amount of GSH was 7.1 ± 0.9 nmol/lens in the HC + GTP group ($p < 0.001$ vs. non-HC group, $p < 0.001$ vs. HC alone group) (Fig. 1). Therefore, GTP partially recovered the HC-induced reduction in GSH level of the lens.

3. Blood glucose level

The blood glucose levels were 185 ± 9 mg/dL in the non-HC group and significantly higher in the HC-alone group (356 ± 29 mg/dL,

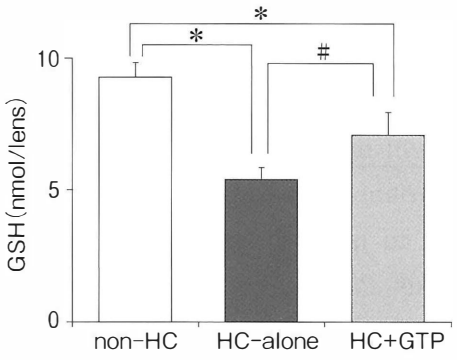

Fig. 1. Effect of HC and GTP on the GSH in the lens
HC decreased lenticular GSH level from 9.2 ± 0.6 nmol/lens (non-HC) to 5.4 ± 0.5 nmol/lens (HC-alone). This decrease was partially recoverd by administration of GTP. The amounts of GSH in lenses were 7.1 ± 0.9 nmol/lens (HC + GTP).
*: $p < 0.001$ vs. non-HC, #: $p < 0.001$ vs. HC-alone (Sheffe's test).

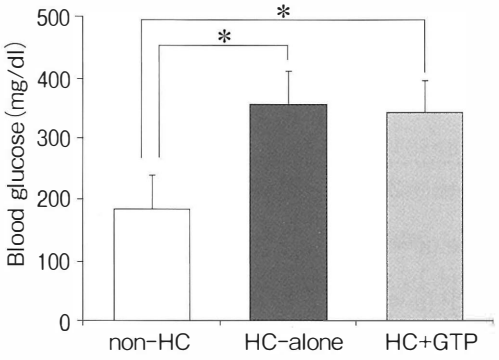

Fig. 2. Effect of HC and AST on the blood glucose level
HC treatment increased the blood glucose level from 185 ± 9 mg/dL to 356 ± 29 mg/dL. The blood glucose level was 341 ± 32 mg/dL in the HC+GTP group. There was no significant difference between the HC-alone group and the HC + GTP group ($p = 0.790$, Sheffe's test).
*: $p < 0.001$ vs. non-HC (Sheffe's test).

$p < 0.001$). The blood glucose level was 341 ± 32 mg/dL in the HC + GTP group. No significant difference was detected between the HC-alone and HC+GTP groups (Fig. 2). Therefore, GTP did not affect the serum glucose level of embryos treated with HC.

IV. Discussion

In this study, we examined whether GTP prevents cataract formation using a steroid-induced chick embryo model of cataracts. We discovered that treatment with GTP significantly suppressed the HC-induced reduction of lens GSH level. This result suggests that the suppression of lens opacity by GTP involved a reduction of oxidative stress.

To date, only one study has reported that tea polyphenols prevent cataracts in vivo. The study reported that lens opacity induced by vitrectomy surgery with silicone oil was prevented by the application of an ophthalmic gel of tea polyphenols to rabbits [17]. However, because this surgery in humans is usually performed along with cataract surgery, it is rarely performed for phakic eye conditions.

The chick embryo model of steroid-induced cataract is a well-established in vivo animal model of the disease [18]. According to previous studies, the cataract that develops is not attributable to local glucocorticoid effects [19]; stimulation of hepatic peroxidation reactions elevates the lipid peroxide levels [20]. The lipid peroxide penetrates the lens via the blood and aqueous fluid and exerts oxidative stress, potentially causing cataract when the oxidative stress level exceeds the inherent antioxidant potential of the lens [21]. Furthermore, glutathione levels in the lens decrease, which enhances the cataract development. Therefore, the chick embryo model of steroid-induced cataract

formation is considered an excellent, easy-to-develop experimental model that readily enables drug treatment and is useful for screening antioxidant drug activity. To date, several antioxidant substances such as vitamin C [22,23], pyrroloquinoline quinone [24], tiopronin [24,25], cysteamine [24,26], and astaxanthin [27] have been reported to suppress cataract formation in this model.

In chick embryos, HC enhances both hepatic peroxidation reactions and hepatic gluconeogenesis [28], and insulin was reported to suppress cataract formation in this model [29]. GTP did not affect blood glucose levels in this study, although some studies have reported that GTP increased sensitivity to insulin, thereby increasing glucose uptake into endothelial cells [30-32]. However, in this study, GTP did not suppress cataract formation by affecting glucose metabolism, and the present dose used might have been too low to affect sugar metabolism. More extensive studies using higher doses of GTP would be necessary to evaluate this speculation.

Oral administration of GTP reportedly leads to its detection in the aqueous humor, resulting in an increased antioxidant potential [33]. Therefore, GTP injected into the air chamber of a chicken egg may be taken up into the blood, subsequently penetrate the barrier of the eye, and exert antioxidant actions. However, it is not clear whether GTP acted in the eye in this study because we did not determine the levels.

In the present study, administration of GTP to a chick embryo model of steroid-induced cataract significantly suppressed the HC-induced lens opacity and recovered the levels of GSH. These results suggest that the GTP-induced suppression of lens opacity involved the reduction of oxidative stress, and this substance is worth further investigation for development as a possible therapeutic option for the treatment of cataracts.

Conflict of interest: The authors have no conflict of interest to declare.

References

1) **McCarty CA**: Cataract in the 21st Century: lessons from previous epidemiological research. Clin Exp Optom **85**, 91-96, 2002.
2) **Taylor HR, West SK, Rosenthal FS, et al.**: Effect of ultraviolet radiation on cataract formation. N Engl J Med **319**, 1429-1433, 1988.
3) **Hiller R, Giacometti L** and Yuen K: Sunlight and cataract: an epidemiologic investigation. Am J Epidemiol **105**, 450-459, 1977.
4) **Christen WG, Manson JE, Seddon JM, et al.**: A prospective study of cigarette smoking and risk of cataract in men. JAMA **268**, 989-993 1992.
5) **West S, Munoz B, Emmett EA, et al.**: Cigarette smoking and risk of nuclear cataracts. Arch Ophthalmol **107**, 1166-1169, 1989.
6) **Hiller R, Sperduto RD, Podgor MJ, et al.**: Cigarette smoking and the risk of development of lens opacities. The Framingham studies. Arch Ophthalmol **115**, 1113-1118, 1997.
7) **Hankinson SE, Willett WC, Colditz GA, et al.**: A prospective study of cigarette smoking and risk of cataract surgery in women. JAMA **268**, 994-998, 1992.
8) **Krishnaiah S, Vilas K, Shamanna BR, et al.**: Smoking and its association with cataract: results of the Andhra Pradesh eye disease study from India. Invest Ophthalmol Vis Sci **46**, 58-65, 2005.
9) **Kelly SP, Thornton J, Edwards R, et al.**:

Smoking and cataract: review of causal association. J Cataract Refract Surg **31**, 2395-2404, 2005.

10) **Jacques PF** and **Chylack LT**: Epidemiologic evidence of a role for the antioxidant vitamins and carotenoids in cataract prevention. Am J Clin Nutr **53**, 352-355, 1991.
11) **Varma SD**: Scientific basis for medical therapy of cataracts by antioxidants. Am J Clin Nutr **53**, 335-345, 1991.
12) **Sies H**: Oxidative stress: from basic research to clinical application. Am J Med **91**, 31-38, 1991.
13) **Guo Q, Zhao M, Li M, et al.**: Studies on protective mechanisms of four components of green tea polyphenols against lipid peroxidation in synaptosomes. Biochim Biophys Acta **1304**, 210-220, 1996.
14) **Noda Y, Anzai A, Mori M,et al.**: Hydroxyl, end superoxide anion radical scavenging activities of natural source antioxidants using the computerized JES-FR30 ESR spectrometer system. Biochem Mol Biol Int **42**, 35-44, 1997.
15) **Anesini C, Ferraro GE** and **Filip R**: Total polyphenol content and antioxidant capacity of commercially available tea (Camellia sinensis) in Argentina. J Agric Food Chem **56**, 9225-9229, 2008.
16) **Nishigori H, Lee JW** and **Iwatsuru M**: An animal model for cataract research: cataract formation in developing chick embryo by glucocorticoid. Exp Eye Res **36**, 617-621, 1983.
17) **Ma X, Guo D, Bi H, et al.**: Protective effects of tea polyphenol ophthalmic gel on lens epithelial cells in rabbits with silicone oil tamponade after vitrectomy. Evid Based Compl Alternat Med, Article ID 832381, 2014.
18) **Nishigori H, Lee JW** and **Iwatsuru M**: An animal model for cataract research: cataract formation in developing chick embryo by glucocorticoid. Exp Eye Res **36**, 617-621, 1983.
19) **Nishigori H, Kosano H** and **Umeda IO**: Inhibition of glucocorticoid-induced cataracts in chick embryos by RU486: a model for studies on the role of glucocorticoids in development. Life Sci **75**, 3027-3033, 2004.
20) **Lee JW, Iwatsuru M** and **Nishigori H**: Alteration of activities of hepatic antioxidant defence enzymes in developing chick embryos after glucocorticoid administration--a factor to produce some adverse effects. J Pharm Pharmacol **50**, 655-660, 1998.
21) **Kosano H, Watanabe H** and **Nishigori H**: Suppressive effects of thyroxine on glucocorticoid (gc)-induced metabolic changes and cataract formation on developing chick embryos. Exp Eye Res **72**, 643-648, 2001.
22) **Nishigori H, Hayashi R, Lee JW, et al.**: Preventive effect of ascorbic acid against glucocorticoid-induced cataract formation of developing chick embryos. Exp Eye Res **40**, 445-451,1985.
23) **Murakami I, Kosano H, Uga S,et al.**: Comparison of lens biochemistry and structure between BSO-treated and glucocorticoid-treated developing chick embryos. Exp Eye Res **63**, 673-681,1996.
24) **Nishigori H, Yasunaga M, Mizumura M, et al.**: Preventive effects of pyrroloquinoline quinone on formation of cataract and decline of lenticular and hepatic glutathione of developing chick embryo after glucocorticoid treatment. Life Sci **45**, 593-598, 1989.
25) **Nishigori H, Hayashi R, Lee JW, et al.**: Effect of MPG on glucocorticoid-induced cataract formation in developing chick embryo. Invest Ophthalmol Vis Sci **25**, 1051-1055, 1984.
26) **Setogawa T, Kosano H, Kayanuma T, et al.**: Preventive effect of SA3443, a novel cyclic disulfide, on glucocorticoid-induced cataract formation of developing chick embryo. Exp Eye Res **58**, 689-695, 1994.
27) **Ishikawa S, Hashizume K, Noshigori H, et al.:** Effect of astaxanthin on cataract formation induced by glucocorticoids in the chick embryo. Cur Eye Res **40**, 535-540, 2015.
28) **Nishigori H, Lee JW, Yamauchi Y, et al.**: Elevation of glucose in ocular compartments of developing chick embryos with glucocorticoid-induced cataract. Exp Eye Res **49**, 515-522, 1989.
29) **Watanabe H, Kosano H** and **Nishigori H**: Steroid-induced short term diabetes in chick embryo: reversible effects of insulin on metabolic changes and cataract formation. Invest Ophthalmol Vis Sci **41**, 1846-1852, 2000.
30) **Anderson RA** and **Polansky MM**: Tea enhances insulin activity. J Agric Food Chem **50**, 7182-7186, 2002.
31) **Wu LY, Juan CC, Ho LT, et al.**: Effect of green tea supplementation on insulin sensitivity in Sprague-Dawley rats. Agric Food Chem **52**, 643-648, 2004.
32) **Potenza MA, Marasciulo FL, Tarquinio M,**

et al.: EGCG, a green tea polyphenol, improves endothelial function and insulin sensitivity, reduces blood pressure, and protects against myocardial I/R in SHR. Am J Physiol Endocrinol Metab **292**, 1378-1387, 2007.

33) **Chu KO, Chan KP, Wang CC, et al.**: Green tea catechins and their oxidative protection in the rat eye. J Agric Food Chem **58**, 1523-1534, 2010.

岩手医誌 69 巻, 5 号（平成 29 年 12 月）265-272 頁.

ステロイド誘発鶏胚白内障モデルにおける緑茶ポリフェノールの効果

前田可奈子[1)], 橋爪公平[1)], 石川清乃[1)],
小笠原聡[1)], 奥野　孟[1)], 清野太郎[1)],
西郡秀夫[2)], 三部　篤[2)], 黒坂大次郎[1)]

[1)] 岩手医科大学医学部，眼科学講座
[2)] 岩手医科大学薬学部，薬剤治療学講座

(Received on January 20, 2017 & Accepted on February 16, 2017)

要旨

本研究では，ステロイド誘発鶏胚白内障モデルを用いて，green tea polyphenols (GTP) の投与により白内障形成が抑制されるかどうかを検討した．

抱卵後 15 日の受精鶏卵の気室部にヒドロコルチゾン (HC)0.5 μmol/egg を投与し，ステロイド誘発鶏胚モデルを作成．HC 投与後 3 時間，10 時間，20 時間後に生理食塩水に溶解した GTP (200 μl/ 回) を同部に投与，生理食塩水を投与する群をコントロール群とした．HC 投与後 48 時間で解剖を行い，摘出水晶体を肉眼にて観察し，水晶体混濁の評価 (1 ～ 5 にスコア化) を行った．また水晶体中の還元型グルタチオン量の測定を行った．

ステロイド誘発鶏胚白内障モデルでは酸化ストレスがその病態に関与していると考えられおり，このモデルに対して GTP を投与すると水晶体混濁が抑制され，水晶体中グルタチオン量の減少が低下した．

このことより，GTP が抗酸化作用により水晶体混濁を抑制したことが示唆された．