Original Article

Luteolin: a novel approach to attenuating the glaucoma via antioxidant defense mechanism

Jianshu Yuan, Yuwen Wang, Xiaoping Xu, Pengyun Wang

Department of Ophthalmology, Ningbo Eye Hospital, Ningbo 315040, Zhejiang, China

Received September 25, 2016; Accepted November 18, 2016; Epub May 1, 2017; Published May 15, 2017

Abstract: The aim of the current investigation was to explore the anti-glaucoma effect of luteolin in the experimentally induced glaucoma. The New Zealand rabbit was used for the current experimental study. Intraocular pressure (IOP) percentage change was estimated after induction the dextrose induced acute glaucoma in rabbit. The rabbit was treated with the luteolin (5, 10 and 20 mg/kg), acetazolamide (5 mg/kg) and normal saline, respectively. Another model such as prednisolone acetate and rabbits were treated with luteolin, acetazolamide and normal saline, respectively for 2 weeks. We also evaluated the antioxidant effect of luteolin via scrutinized the content of glutathione and glutamate in the experimental rabbits via using the ELISA assay. Histopathological observation of the ciliary bodies was also evaluated. Luteolin significantly (P≤0.001) inhibited the intraocular pressure in both acute and chronic glaucoma model, preserved glutamate and glutathione content. Histopathological studies showed the less inflammatory infiltration in the luteolin and acetazolamide treated group rabbits as compared to the normal group rats. The result revealed that luteolin proved the ocular hypotensive, neuroprotective and antioxidant effect, which consequently underscore its possible use as an anti-glaucoma drug with additional experimental study.

Keywords: Acetazolamide, luteolin, glutathione assay, anti-glaucoma

Introduction

Glaucoma is considered as the dreadful disease, its recorded as the 2nd most common induce the blindness and is leading to induce the irreversible blindness worldwide [1, 2]. Glaucoma is a heterogeneous group disease inducing the multiple factors such as rise the vascular dysregulation and intraocular pressure (IOP). Both factors strongly sharethe initial damage in the disorder by disturbance of the optic nerve microcirculation (lamia level), obstructing the axoplasmic flow in the retinal ganglion cell axons at the lamina cribrosa and changing the connective tissue and laminar glial [3].

Several evidences have confirmed that, further injury such as excitotoxicity will be induced by glycine or glutamate that is freed from damaged neurons and induces the oxidative stress [4]. The treatment available for the glaucoma, blindness still available to nearly 10% of World population. The primary form of the glaucoma is primary open angle glaucoma (POGA), found with or without warning symptoms, primary developing stage of glaucoma.

Flavonoids are universally distributed and widely considered as secondary metabolites of plants with various pharmacological properties. Flavonoids showed anti-viral, anticancer, anti-oxidant, anti-inflammatory and anti-parasitic activity. As other flavonoids, luteolin (3,4,5,7-tetrahydroxyflavone), is often found in the various vegetables and fruit materials in the form of glycosides and has contributed as the antioxidant property in various disease. It showed anti-inflammatory, anti-allergic and anti-cancer activity.

Since luteolin has anti-inflammatory, anticancer, antioxidant and free radical scavenging potential, we assume that the luteolin might possess the protective effect against the glaucoma. In the current study, we have used the rabbit model to induce the glaucoma via dextrose and prednisolone, to scrutinize the beneficial effect of luteolin and find out its possible mechanism of action.
Experimental

Chemicals

Ocular hypotension was induced by using the suspension of prednisolone acetate ophthalmic. Proparacaine was utilizing as the local anaesthetic in eye during IOP measurements. Acetazolamide was used as the anti-glaucoma drug.

Experimental study

White New Zealand rabbits (1±0.3 kg) were used for the current experimental study. The rabbit was stored in the single aluminum cages with soft wood shavings as bedding, under standard laboratory conditions (60-70% relative humidity, 25±2°C temperature and 12 h dark-light cycle). The rabbits were received the normal diet pellet and water ad libitum. The current experimental study was approved form the Institutional animal ethical committee. The experimental study was performed according to the principles of the use and care of laboratory animal and the association for research in vision and ophthalmology statement for use of animals in ophthalmic and vision research.

An acute glaucoma model

Schiotz indentation tonometer was used for the estimation of basal IOP in each rabbit eye, which was further calibrated via an open manometric calibration procedure as mentioned elsewhere [5]. The whole experiment was performed with care to avoid the attachment of nictitating membrane from coming under the base of tonometer. Two weights such as 5.5 g and 10 g were used for recording the tension and mean of the two readings was estimated. The animals were divided into 5 groups and each group contains the 6 rabbits. Group 1-3 received the leuteolin 5, 10 and 20 mg/kg, respectively. While groups 4 and 5 received the acetazolamide (5 mg/kg) and saline (10 mL/kg) respectively. The above discuses rabbits received the oral treatment once a day and the quantity of the oral treatment was not more than the 1 mL. After receiving the oral administration of various drugs and saline, the rabbits were intravenously treated with the dextrose (5%) via marginal ear vein. The determination of IOP was done after the 20 and 120 min. the IOP change was calculated using the following formula

\[ \text{IOP changes (\%)} = \frac{\text{IOP}_t \cdot \text{IOP}_o}{\text{IOP}_o} \times 100 \]

Where IOP, denotes the ocular tension at different times (after dextrose treatment) and IOPo is the ocular tension at zero times (before dextrose treatment).

Estimation of glutamate in vitreous humour

Glutamate assay kit was used for the estimation of glutamate concentration in vitreous humour in experimental rabbits. The vitreous humour was collected in the eppendof tubes after assessing it via a sclera puncturing at the lateral canthus. The collected vitreous was sonicated in perchloric acid (0.2 M) containing EDTA (0.1%) and Na2S2O5 (0.1%). The homogenate was homogenized via using the centrifuge at 20000 rpm for 4 min at 4°C. The supernatant was used for the estimation the concentration of glutamate. The determination of glutamate in the standard and samples via using the manufacturer instruction.

Estimation of hypotensive effect of leuteolin in chronic glaucoma model

The induction of ocular hypertension was estimated in the rabbit, after estimation of IOPs, prednisolone acetate (1%) was used for the induction the ocular hypertension in each eye of rabbit, twice daily for 21 days. The rabbits having enhance IOP (50%) with one or more of the following clinical signs: fixed dilated pupils, limbal, buphthalmic eyes (bulging eyeball) and papillary reaction.

The ocular hypotensive effect of leuteolin was determined via dividing the rabbits into following groups. Group 1-3 treated with the leuteolin 5, 10 and 20 mg/kg, respectively. Group 4 (acetazolamide) used as positive control and group 5 (saline) served as the negative control. The treatment was continued till the 2 weeks and the intraocular pressure was estimated of the each eye of the rabbit.

Estimation of glutathione in aqueous humor

Glutathione kit was used for the estimation of aqueous humour of experimental rabbits. The animal was euthanized and needle was used
Anti-glaucoma potential of leuteolin

Figure 1. Time-course curves and areas under the curve for the acute glaucoma study. Time-course curves (A, C) and areas under the curve (B, D) for the effects of pretreatment with 5, 10 and 20 mg/kg of leutolin, 5 mg/kg Acetazolamide (ACET), and 10 mL/kg normal saline (NS) on Dextrose-induced ocular hypertension of the right eye (A, B) and left eye (C, D) in New Zealand White Rabbits. Values plotted represent mean ± SEM (n=5). ***P≤0.001, ANOVA followed by Dunnett’s post-hoc test.

Figure 2. Time-course curves and areas under the curve for the chronic glaucoma study. Time-course curves (A, C) and areas under the curve (B, D) for the effects of treatment with 5, 10 and 20 mg/kg of leutolin, 5 mg/kg Acetazolamide (ACET), and 10 mL/kg normal saline (NS) on steroid-induced ocular hypertension of the right eye (A, B) and left eye (C, D) in New Zealand White Rabbits. Values plotted represent mean ± SEM (n=5). ***P≤0.001, **P≤0.01, *P≤0.05. ANOVA followed by Dunnett’s post-hoc test.
Anti-glaucoma potential of leuteolin

for puncturing the anterior chamber. The collected aqueous chamber was stored in the sterile tubes. Triethanolamine (4 M) and metaphosphoric acid was used for deproteinated the aqueous humour according to the manufacture’s instruction. The aqueous humour (50 μL) and standard were transferred into 96 well plates, incubated at dark room and estimated the absorbance at 405 nm and all the data performed in triplicates.

**Histopathological assessment**

The enucleated eyes of the animals were fixd in 10% phosphate-buffered formaldehyde, and embedded in paraffin for histopathological assessment. Sections were made and stained with haematoxylin and eosin and alcian blue [6-8]. Sections were fixd on glass slides for microscopic examination by a specialist pathologist at the Pathology Department of the Komfo Anokye Teaching Hospital, Kumasi, Ghana.

**Histopathology study**

The eye of the rabbits (enucleated) was fixd in the phosphate buffered formaldehyde (10%), and embedded in paraffin for histopathological changes. Heamatoxylin and eosin was used for the stained the sections.

**Statistically analysis**

All the data was analyzed using the Dunnett’s multiple comparisons test using the GraphPad prism. All the data was expressed as the mean ± standard error of mean and P<0.05 was considered as significant.

**Results**

**Figure 1** showed that the leuteolin significantly (P≤0.001) protected the expected increase the IOP in dextrose induced ocular hypertension as compared to normal saline treated rabbit. The acetazolamide and leuteolin treated group rabbits did not show any considerably reducing effect in the experimental rabbits. On the other steroids induced ocular hypertension model leuteolin treated group demonstrated the inhibition of IOP on the right and left eyes of rabbit as compared to normal group. We also compared the effect with the acetazolamide group (**Figure 2**).

**Glutathione and glutamate content in aqueous humour**

**Figure 3A** showed the content of glutathione in aqueous humour, acetazolamide and leuteolin group raabbits demonstrated the significantly (P≤0.001) declined the oxidative stress via preserving endogenous glutathione content.
Anti-glaucoma potential of leuteolin

Figure 3B demonstrated the content of glutamate in the vitreous humor, acetazolamide and leuteolin induced a significant inhibition of excitotoxin in the vitreous humor in the ocular hypertensive rabbits.

Histopathology examination

The anterior chamber indicates the alteration of the morphological changes in the ciliary bodies of leuteolin and acetazolamide treated group rabbits. Moreover, the modulation sigh of the histopathology characterized by mononuclear infiltration into the ciliary body (Figure 4).

Discussion

Glaucoma is defined as the ocular disorders with multiple-factorial induce via a clinical optic neuropathy with or without enhance the intraocular pressure. Few research claims that the IOP is the best approach to treat the glaucoma. Experimentally induce glaucoma is a model, which is similar to the human condition, and it is very effective to understand the clinical condition and pathophysiological condition of the disease and also effective in find out the potential anti-glaucoma agent [9].

Two model (dextrose and water loading) was used for induce the glaucoma in the animals. Intravenously administration of dextrose model having the advantage over the water loading model. Intravenously administration of dextrose inhibit the serum osmolarity followed by sugar has been removed from the circulation. The reduction of the serum osmolarity starts the circulation of water into the eye thus enhancing the IOP. Dextrose control rabbit treated with the leuteolin confirmed the preventive effect against the enhancement of IOP content as compared to the normal treated group animals, confirming that the leuteolin could be acting via enhancing outflow facility or reduction of aqueous humor production. Few researchers showed relationship between expansion of glaucoma and systemic blood pressure. In case of hypotension, enhanced blood pressure, increase the blood circulation to the optic nerve which causes the glaucoma via ischemic tendencies. Another fact confirmed that the hypotension increased the risk factor of glaucoma, further clinical studies needed to find out the protective effect of leuteolin in the treatment of glaucoma and in the current experimental studies we also reported the decreased blood pressure.

Several studies confirmed that the free radical play a significant role in the pathophysiology of glaucoma. Free radical starts the generation of oxidative stress which starts the deficient in endogenous antioxidant defense system. It is already confirmed that the increased loss of visual field and IOP affect the oxidative DNA damage and also affecting the trabecular meshwork cells and outflow facility. Glutathione found in the significant proportion in aqueous humour and also play a significant role in the protection from the system against oxidative stress aggravated diseases. The antioxidant status of biological samples is useful as a marker of oxidative stress. The leuteolin treatment conserved the endogenous humour glutathione levels, which confirm that the leuteolin not only effective against decreasing IOP but also produce the protection against oxidative injury and progression of glaucomatous neurodegeneration. The result confirmed that the
leuteolin targets the mitochondrial cell of trabecular meshwork in exerting its effect [10].

The anti-glaucoma effect of leuteolin was further evaluated on the chronic model of ocular hypotensive. Corticosteroid induced ocular hypotension model bears impression of POAG, and is exemplified via aqueous outflow obstruction, optic nerve cupping and visual field effects. Leuteolin treatment declined the IOP induced by steroids pretreatment in rabbits. Recent evidence confirmed that the POAG, as modeled by corticosteroid induced ocular hypertension in experimental rabbits, the disease involved in the optic nerve head, trabecular meshwork, lateral geniculate nuclei, and visual cortex. Repeated intake of steroids trigger the oxidative stress resulting induce the apoptosis trabecular meshwork cell humour and antioxidant system. The result confirmed that the leuteolin exerting the ocular hypotensive effect via protection of structural integrity and enhancing the aqueous outflow or both.

Conclusion

The result revealed that the leuteolin exhibit ocular hypotensive, neuroprotective and antioxidant effects hence, it could be used as a novel approach to treat the glaucoma disease.

Acknowledgements

The present research was supported by the Department of Ophthalmology, Ningbo Eye Hospital, China.

Disclosure of conflict of interest

None.

Authors’ contribution

Yuan, Wang, Xu and Wang design and performed the experimental study. Wang and Xu analysis the data and edit the manuscript. All authors read and approved the final manuscript.

References


