Effect of Vitamin C on intraocular pressure: An experimental study in rabbits

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الخلاصة:
عشرون أرنبا (نوع الأبيض النيوزلندي) استخدمت في هذه الدراسة ثم رفع ضغط العين اليمنى للأرانب وبعد ذلك قسمت الأرانب عشوائيا إلى أربع مجاميع (٥ كل مجموعة) ثم عولجت الحيوانات بالماء المقطر (المجموعة الأولى) لمدة عشرة أيام تم قياس ضغط جحر العين قبل وبعد استخدام الأدوية المذكورة وكانت النتائج كالأتي:
بالمقارنة مع المجموعة الأولى أحدث التايمولول انخفاضا ملحوظا في ضغط العين وكذلك فيتامين (سي).
وعند استخدام التايمولول مع فيتامين (سي) انخفض ضغط جحر العين انخفاضا ملحوظا جدا وأكثر من استخدام أي من الأدوية على حده ومن هنا نستنتج إن فيتامين (سي) لديه أهمية ملحوظة في خفض ضغط العين.

Abstract

Background: Glaucoma is the second leading cause of blindness, after cataract. Growing evidence suggested that the oxidative damage might be a relevant target for both glaucoma prevention and therapy.

Objective: The present study was undertaken to clarify the Intraocular pressure (IOP) lowering effect of vitamine C topical application.

Materials and methods: A total of 20 male of White New Zealand Rabbits were enrolled in the study. Ocular hypertension was induced (by hyroxymethyl cellulose) in the right eye of all rabbits and the animals randomized into four groups, five rabbits each. The animals then treated with eye drop of D.W (Group 1), timolol 0.5% (Group 2), vitamin C 0.5% (Group 3) and a combination of timolol 0.5% and vitamin C 0.5% (Group 4) three times daily for 10 days. IOP was measured before and after drug treatment.

Results: Compared to group 1, Timolol 0.5% (15.66 ± 0.31 vs. 25.2 ± 0.29 mmHg,) and vitamin C 0.5% (17.57 ± 0.42 vs. 25.2 ± 0.29 mmHg) caused a significant ($p < 0.05$) decrease in IOP. Further, a combination of Timolol 0.5% and vitamin C 0.5% produced highly significant ($P < 0.01$) fall in IOP.

Conclusion: We can concluded that vitamin C has a significant IOP lowering effect, possibly due to antioxidant properties

Key words: Glaucoma, Oxidative stress, Vitamine C

Introduction

Glaucoma is a slow progressive degeneration of the retinal ganglion cells and the optic nerve axons, leading to increasing deterioration of the visual field. If untreated, the condition can lead to irreversible blindness [(1)]. Glaucoma is the second leading cause of blindness, after cataract. Worldwide, its estimated that about 66.8 million people have visual impairment from glaucoma, with 6.7 million suffering from blindness [(2)].

Increase intraocular pressure (IOP) is the major risk factor for primary open angle glaucoma. In addition other factors such as alteration in nitric oxide (NO) metabolism,

vascular alteration and oxidative damage caused by reactive oxygen species (ROS) are also involved [3].

Oxidative stress may contribute to glaucoma etiology and progression. Much evidence indicate that oxidative stress play a fundamental pathogenic role by reducing local antioxidant activities, inducing outflow resistance and exacerbating the activities of superoxide dismutase and glutathione peroxidase in glaucomatus eye. In addition, hydrogen peroxide induces rearrangement of trabecular meshwork (TM) cells and integrity [4,5]. Furthermore, oxidative stress contributes in the alteration of NO homeostasis. NO is involved in the regulation of trabecular out flow and thus maintaining the normal function of the aqueous flow pathway[6]. Therefore, the oxidative damage might be a relevant target for both prevention and therapy.

Ascorbic acid, water-soluble vitamin (vitamin C), is a significant component of the aqueous humor and its concentration is more than 15 times higher than that in plasma[7,8]. Recent studies suggested that vitamin C may play an important role in maintaining trabecular outflow pathway by antioxidant function through removing ROS [9]. Moreover, vitamin C was also known to reduce IOP by the depolmerization of the TM cells hyaluronic acid component [10]. Therefore the present study was undertaken to clarify the IOP lowering effect of vitamine C topical application.

Material and method

Preparation of animal

A total of 20 male of White New Zealand Rabbits (Oryctologus cuniculus), aged eleven months with body weight of (1.5-2 kg) were enrolled in the study. The animals were kept in the animal house of Al Qadisiyah College of Medicine at suitable temperature and a 12 h light/ dark cycle. The animals had free access to water *ad libitum*.

Desgin of the study

After two weeks of adaptation, ocular hypertension was induced in the right eye of all rabbits. Two days later, the animals randomized into four groups, 5 rabbits each. The animals then treated with D.W (Group 1), timolol 0.5% (Group 2), vitamin C 0.5% (Group 3) and a combination of timolol 0.5% and vitamin C 0.5% (Group 4). The drug treatment was performed as eye drop instillation into the right eye, 3 times daily for 10 days.

Induction of ocular hypertension

Animals were anesthetized by intramuscular administration of ketamine hydrochloride 20 mg/kg (Oboi Laboratories, India). Then under sterile condition, induction of ocular hypertension was carried out by injection of 0.4 ml of (2% w/v) hydroxypropyl methylcellulose (HMC) (Focus vision care-United States pharmacopoeia) into anterior chamber of the right eye [11,12].

Preparation of the drugs used in the study

Timolol 0.5% eye drop (Timolyre, Delta Pharma., Syria) purchased from the pharmacy while vitamin C 0.5% eye drop was performed locally by using the following agents: vitamin C 0.5 g (Emessa Labs /Homs – Syria), benzalkonium chloride 1% (w/v) 1 ml (SDI), sodium chloride 0.44 g (Riedel – De Haen Ag seelze – Hannover), ethanol 70 % 1ml (Emscope Laboratory Ltd) and phosphate puffer 100 ml [13].

Measurement of IOP

By using Sehiotz tonometer, IOP was measured at the beginning of the experiment (baseline), 2 days after HMC injection (ocular hypertension baseline) and 10 days after drug treatment.

Statistical methods

The data was expressed as mean ± standard error of mean (SEM) unless otherwise stated .Statistical analysis was carried out using paired ț test and ANOVA. Significant difference was set at (P = 0.05) .P value less than 0.05 level of significant was consider statistically significant [14].

Results

1-Effect of injecting HMC into anterior chamber of the right eye:

Compared to baseline value, injecting HMC into anterior chamber of the right eye resulted in significant ($P < 0.01$) rise in IOP (11.87 ± 0.25 vs. 24.33 ± 0.19 mmHg) (figure 1).

2-Effect of drug treatment on IOP:

Compared to ocular hypertension baseline value (OHB), there weren't any statistically significant changes ($P>0.05$) in the IOP (24.33± 0.19 vs. 25.2± 0.29) of the D.W treated group. This group considered as negative control for further comparison (figure 2).

Timolol 0.5% caused significant decrease ($p < 0.05$) in the IOP (15.66 ± 0.31 vs. 25.2 ± 0.29 mmHg) as compared to negative control group (Group1) (figure 2).

Compared to negative control group, instillation of vitamin C 0.5% eye drop resulted in significant ($p < 0.05$) fall in the IOP (17.57 ± 0.42 vs. 25.2 ± 0.29 mmHg) (figure 2).

In comparison to timolol 0.5% and vitamin C 0.5% treated groups, a combination of these two drugs resulted in highly significant ($p< 0.01$) decrease in the IOP (13.92 ± 0.35 mmHg) (figure2).

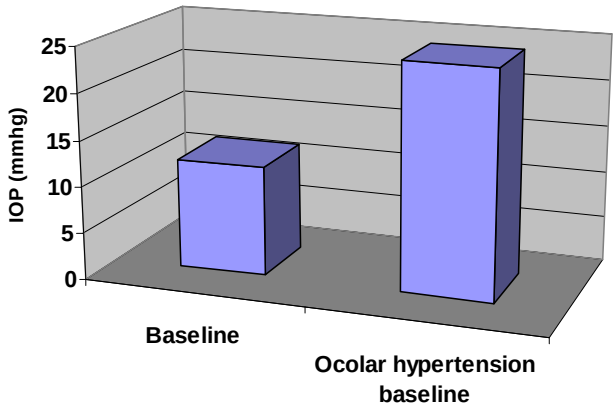

Figure (1): Mean changes in IOP (mmhg) 2 days after hyroxymethyl cellulose injection as compared to baseline value.

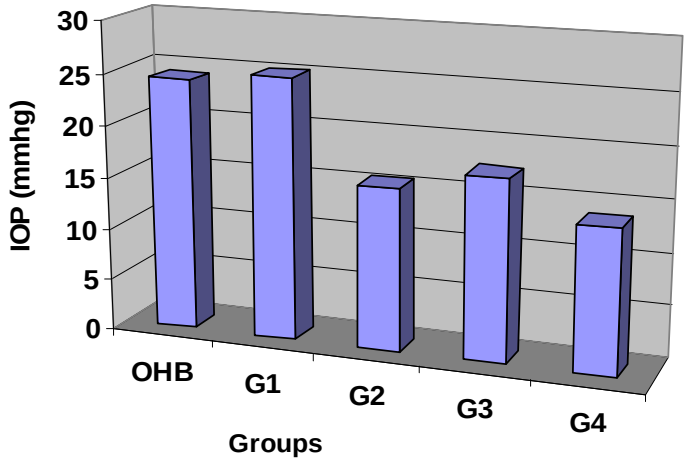

Figure (2): Mean changes in IOP (mmhg) after 10 of drug treatment, as compared to ocular hypertension baseline (OHB).

Discussion

Several mechanisms involved in the pathogenesis of glaucoma; increase aqueous humor formation and viscosity and obstruction of the drainage channel with subsequent outflow resistance [15]. Recent evidences stated that oxidative stress that exceeds the capacity of TM cells for detoxification could damage the TM cells with subsequent alteration of aqueous outflow resistance and development of glaucoma [5]

The present study showed that intraocular injection of HMC caused significant rise in the IOP as compared to baseline values .These findings are consistent with the reports of **Daniel 1983, William 1999, and Bahaa 2006** [14,16,17]. Such experimentally induced ocular hypertension is possibly explained by increasing the viscosity of aqueous humor and obstructing the drainage channel [11,12].

In comparison with negative control, this study demonstrated that timolol 0.5% significantly decreased IOP. These demonstrations agreed with the findings of **Brooks and Gillies 1992 and Zimmerman 1993**. This reduction in the IOP was expected due to β – blocking effect of timolol which decrease aqueous humor formation [18, 19].

In the present study, we observed that topically applied vitamin C 0.5% resulted in significant fall in the IOP. In addition combination treatment of vitamin C 0.5% and timolol 0.5% significantly reduced IOP more than either drug alone**. Paterson G, 1996 and Kim JW, 2005** reported that oral vitamin C may play an important role in maintaining trabecular outflow pathway [6,10]. No other studies yet available and to our best knowledge, this is the first study of the effect of topical vitamin C on glaucoma.

The possible explanation for the IOP lowering effect of vitamin C is due to reduction in the viscosity of hyluronic acid in the trabecular meshwork so enhancing the aqueous humor drainage process. Such decrease in the viscosity is possibly attributed to the anti oxidant property of vitamin C [6,7,9,10] .Moreever the synergistic β – blocking effect of timolol and the antioxidant property of vitamin C are the possible contributors for the favorable result of combination of these agents. Thus we can conclude that vitamin C has a significant IOP lowering effect, possibly due to antioxidant properties. Further studies recommended to measure oxidative stress parameters in the eye tissue.

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