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Effect of oral administration of vitamin C on human aqueous humor ascorbate concentration

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KEY WORDS aqueous humour; ascorbic acid; plasma; cataract extraction; high pressure liquid chromatography; hydroquinones

ABSTRACT

AIM: To study oral administration of vitamin C on human aqueous humour ascorbate concentration. **METHODS**: High performance liquid chromatography (HPLC) coupled with electrochemical detector (ECD) was used. The effect of oral administration of various doses of ascorbic acid, 0 (control), 1.0, 1.5, 2.0, 3.0, and 5.0 g, on its concentration in aqueous humour, obtained from volunteer cataract patients was studied. **RESULTS**: The concentration of ascorbic acid in aqueous humour of control group (without administration of vitamin-C tablet or drug containing ascorbic acid was (254 ± 119) $mg \cdot L^{-1}$. This study revealed that the administration of 2.0 g of ascorbic acid saturate the aqueous humour and further increase in the dose (3.0 g and 5.0 g) did not increase its concentration in aqueous humour, although its concentration was increased in plasma. **CONCLUSION**: Oral administration of 2.0 g of Vc is sufficient to saturate the aqueous humour where it may be helpful in controlling the intra-ocular pressure.

INTRODUCTION

The concentration of vitamin C (Vc) was 10 – 15 times higher in aqueous humour than its concentration in plasma[1,2] where it is actively transported by the iris-ciliary body into the aqueous humour[3]. In the eye Vc protects the retina[4] and lens[5] from damage caused by light-induced free radicals. Under certain conditions Vc produces H_2O_2, which causes the depolymerisation of hyaluronic acid[4]. Following oral administration (two times a day) or ophthalmic solution (three times a day) of Vc to the human cornea, a significant fall in steroid induced intraocular pressure (IOP) was observed[6]. Thus, it is possible that high concentrations of ascorbic acid in aqueous humour may be a factor in the maintenance of normal IOP. In the present study the effect of oral administration of Vc tablets on the concentrations of Vc in aqueous humour, plasma, and serum was investigated. The aim of the present work was to find that whether it is possible to achieve the higher concentration in human aqueous humour, which may be required for the depolymerization of hyaluronic acid in eye.

MATERIALS AND METHODS

Chemicals Ascorbic acid, hydroquinone, cetyltrimethylammonium bromide, metaphosphoric acid, sodium acetate, and glacial acetic acid were from Aldrich Chemical Co, Gillingham, Dorset, UK. HPLC-grade water and acetonitrile were from Rathburn Chemical Co, Walkerburn, Peebleshire, UK. Trichloroacetic acid was obtained from Sigma Chemical Co, Dorset, UK.

Human aqueous humour After obtaining the informed consent, the samples of human aqueous humour and blood were obtained from volunteer patients, either sex, 52 – 92 a, undergoing cataract extraction. After the routine preoperative preparation,

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a sliding peripheral corneo-scleral incision was made with a Wheeler knife and aqueous humour (30 – 200 μL) was aspirated into a tuberculin syringe through a 25-gage needle. Then it was transferred to a sample tube containing *m*-phosphoric acid solution (0.1 mol·L^{-1}; 200 μL) and stored at – 20 ℃. Prior to analysis the volume of aqueous humour in each sample was measured with a microsyringe (100 μL).

Plasma and serum Blood samples (3 – 4 mL) were collected from the same patients with a tuberculin syringe during cataract extraction between 9 – 12 am. Samples were then immediately transferred to 2 sample tubes; one containing heparin and the other without any anticoagulant. The former blood samples were centrifuged at 3000 × *g* for 10 min. The other blood samples were kept in the dark for about 15 min, then stirred with a stainless steel loop wire and centrifuged at 3000 × *g* for 10 min. The plasma or serum (0.5 mL) was transferred to a tube containing *m*-phosphoric acid (0.1 mol·L^{-1}; 0.5 mL) and stored at – 20 ℃ until required for analysis.

Preparation of samples Stock solutions of Vc and hydroquinone (each 1 g·L^{-1} in water) were prepared each day. Prior to analysis all samples were defrosted at room temperature (20 ℃) in the dark. The trichloroacetic acid solution (3 %; 200 μL) was added to human aqueous humour samples (50 μL), which had been preserved with *m*-phosphoric acid. The samples were then centrifuged at 3000 × *g*; 1 μg of hydroquinone was added to the supernatant (100 μL), which was then diluted to 1 mL with mobile phase. Then 50 μL of sample was injected into the HPLC for analysis. The trichloroacetic acid solution (3 %; 200 μL) was added to human plasma and serum samples (0.50 mL). The samples were then centrifuged at 3000 × *g*; 1 μg of hydroquinone was added to the supernatant (500 μL), which was then diluted to 1 mL with mobile phase. Then 50 μL of sample was injected into the HPLC for analysis.

Instrumentation A Hewlett-Packard-1082 HPLC system equipped with an auto-sampler, dual pumps, and a variable wavelength UV detector was used for the analysis. The instrument was fitted with a 5 μm Spherisorb ODS-1 reversed phase column (100 × 4.6 mm id) which was protected with an SGE ODS-1 guard cartridge system (Burke Electronics, Glasgow). The column eluent was monitored with an LC-4A amperometric detector (Bioanalytical Systems). The detector was turned to the oxidation mode and the potential was adjusted to 0.6 V versus the Ag/AgCl reference electrode. Oxygen-free nitrogen gas was used for the operation of the pneumatically driven auto-sampler. The mobile phase was prepared by dissolving sodium acetate (0.08 mol·L^{-1}) and cetyltrimethyl-ammoniun bromide (1.0 mmol·L^{-1}) in acetonitrile-water (5 : 95, vol/vol); the pH of the solution was adjusted to 4.2 with glacial acetic acid. A flow rate of 1 mL·min^{-1} was used.

HPLC with an electrochemical detector (ECD) was selective and highly sensitive method for the analysis of ascorbic acid in plasma and aqueous humour[7].

Calibration curve Solutions containing hydroquinone (1 μg) and varying amounts of ascorbic acid (0.5 – 10 μg) in mobile phase (1 mL) were prepared. These solutions were then injected into the HPLC. The instrumental response was found to be linear over the range of 0.5 to 10 mg·L^{-1}; $r = 0.998$; $\hat{Y} = 0.025 + 0.024X$.

Precision and repeatability The precision of the method was determined by injecting 5 replicates of the same sample of the diluted bovine aqueous humour containing 246.4 mg·L^{-1} of Vc; the residual standard deviation (RSD) was 1.49 %. The reproducibility was studied on 5 separate analyses of the same sample of bovine aqueous humour containing Vc 311.8 mg·L^{-1}; the RSD value was 1.63 %. The RSD values for the precision and repeatability of the method were below 2 %, therefore it is suitable for the analyses of Vc in biological samples; particularly in blood samples, where the concentration of ascorbic acid is very low compared to the aqueous humour.

The effect of oral administration of Vc on its concentration in human aqueous humour The effect of *po* of Vc tablets (500 mg) on its concentration in human aqueous humour and plasma were studied in volunteer patients undergoing cataract surgery. Vc 0, 1.0, 1.5, 2.0, 3.0, and 5.0 g was administered orally to the cataract patients, the night before and in the morning on the day of operation.

Statistical calculations Data were presented as $\bar{x} \pm s$ and compared with Newman-Keuls Range Test. All the statistical calculations were carried out using Minitab, a statistical package.

RESULTS AND DISCUSSION

HPLC-ECD trace of ascorbic acid in human aqueous humour in comparison with a trace obtained by the injection of a standard mixture containing Vc 2 mg·L^{-1} and hydroquinone 1 mg·L^{-1} in the mobile phase were shown in Fig 1.

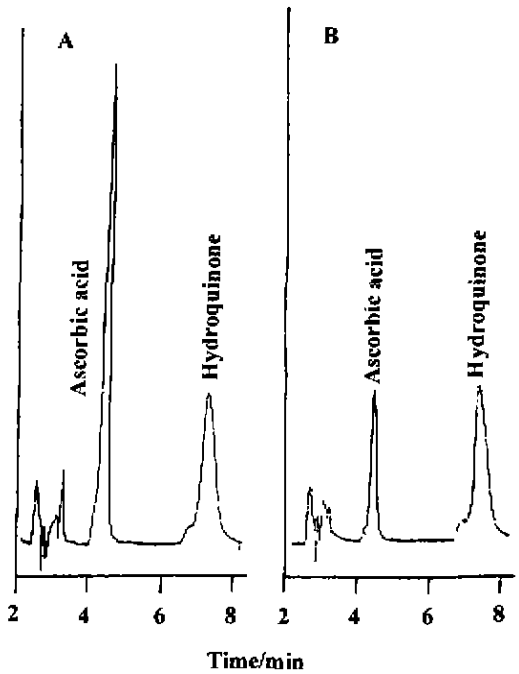

Fig 1. HPLC-ECD tracings of A) Vc from human aqueous humour; B) mixture of Vc 2 μg and hydroquinone 1 μg.

Effect of *po* Vc on its concentrations in human aqueous humour, plasma and serum

The concentrations of Vc in the aqueous humour, plasma and serum of 10 control volunteers were (254 ± 119), (4.5 ± 3.6), and (4.4 ± 3.6) mg·L^{-1}, respectively. The mean concentrations of Vc of control patients was in good agreement with its earlier measurements in aqueous humour[2,7,8], serum[2], and plasma[8] in cataract patients.

Following the oral dose of Vc 1 g, its concentration in aqueous humour was slightly higher than that of the control group but the difference was not significant ($P > 0.05$). However, its concentrations in serum and plasma were higher than those of the unloaded patients. One patient was excluded from the data due to a very low level of ascorbic acid in aqueous humour, 41 mg·L^{-1} (Tab 1).

Tab 1. Concentration of Vc (mg·L^{-1}) in human aqueous humour, plasma, and serum after *po* Vc. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$ *vs* control.

Vc/ mg·kg^{-1}	Patients	Aqueous humour	Plasma	Serum
0 (Control)	10	254 ± 119	4.5 ± 1.1	4.4 ± 1.4
1.0	10	270 ± 62[a]	9.1 ± 1.0[b]	6.0 ± 1.6[b]
1.5	7	526 ± 125[b]		
2.0	19	713 ± 93[b]	13.1 ± 2.0[b]	7.4 ± 1.7[b]
3.0	8	799 ± 94[b]	27 ± 5[b]	15 ± 4[b]
5.0	4	571 ± 88[b]	72 ± 18[b]	

Oral administration of Vc 2 g raised the mean concentration of Vc as follows; in aqueous humour by more than two-fold, in plasma about three-fold, and in serum about two-fold, in comparison with the control group (Tab 1). In serum lower concentrations of ascorbic acid may be due to its degradation when blood was kept for clotting. With further increase in the dose of Vc (3 g and 5 g), the increase in the concentration of Vc in aqueous humour was not significant ($P > 0.05$) in comparison with the concentrations following ingestion of Vc 2 g. Following a *po* Vc 5 g, its mean concentration in aqueous humour was lower than that following the Vc 2 g but still it was within the range after Vc 2 g and 3 g. The concentrations of Vc in plasma and serum were increased significantly ($P < 0.05$) and consistently by the higher doses of Vc 3 and 5 g. Assessment of whether or not there was any significant difference between the various doses of Vc was performed by statistical analysis using the Newman-Keuls Range test.

To investigate whether or not a dose lower than 2 g ascorbic acid was required to saturate the system for transporting ascorbic acid into aqueous humour; samples of aqueous humour, plasma and serum were collected following *po* of 1.5 g of Vc tablets (Tab 1). The concentration of Vc in aqueous humour was 526 mg·L^{-1}, which was higher than those of control patients and that following 1 g *po* Vc, but it was lower than that in aqueous humour following Vc 2 g.

These findings showed that the *po* Vc 2 g caused saturation of the mechanism whereby it is transported into aqueous humour. The wide range of concentration

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of Vc in aqueous humour observed in both control group and these body fluids following *po* (eg 2 g) may be due to one or more of the following factors.

a) Variation in the length of the time between the *po* Vc tablets and the collection of samples; the peak concentration of Vc in plasma occurred 3 h after its *po*[9].

b) The diet of the patients

c) The effect of other drug(s) used during the course of treatment

In human, the supplementation of Vc 2 g (4 × 500 mg daily) for 2 – 4 wk increased its concentration in aqueous humour from 182.6 mg · L^{-1} to 212.5 mg·L^{-1}; this was accompanied by an increase in the concentration of Vc in plasma from 7.0 mg · L^{-1} to 14.0 mg·L^{-1}[9]. In this case a large increase in the concentration of Vc in aqueous humour was not seen might be due to its administration in divided doses.

The reduction of steroid induced IOP following the topical application of Vc[8], increase in the out-flow of aqueous humour after the perfusion of Vc solution (0.1 – 0.4 %) into the bovine eye[8], and low concentrations of Vc with the higher concentration of the copper, in some glaucoma patients[10] shows its potential role in the maintenance of the IOP. Vc may be involved in the depolymerisation of the hyaluronic acid, which increases the IOP when used during the extra capsular cataract extraction (ECCE) surgery[11,12]. Depolymerisation of hyaluronic acid by autooxidation of Vc may involve the free radical reaction[13]. Copper and iron ions in the presence of Vc enhanced the depolymerisation of hyaluronic acid[14]. Thus the increase in IOP after ECCE surgery where hyaluronic acid has been used may be due to its slower degradation in anterior chamber of eye. Also, in open-angle glaucoma patients where the concentration of Vc was very low, the higher IOP may be due to the slower rate of depolymerisation of hyaluronic acid, which was accumulated at trabecular meshwork and hindered the outflow of aqueous humour[15].

These studies, including the present findings, indicate that there is an increase in the concentration of Vc in aqueous humour and plasma after *po* of various doses to human. However, the entry of Vc into the aqueous humour may be limited by saturation of the enzyme system. The supplement of Vc tablets to the glaucoma patients or to the cataract patients undergoing ECCE surgery may help in the controlling the IOP. The results of the current study indicate that an oral dose of Vc 2 g was sufficient to produce this effect and it afforded a two-fold rise in the concentration of Vc in the aqueous humour. At the same time this produced a larger increase in the concentration of Vc in the plasma relative to that of the control subjects. The wide range of concentrations of Vc in the plasma of members of the control group may indicate that some people are deficient in Vc although concentrations in the blood do not necessarily reflect the concentrations in tissues. It is notable that the range of concentrations of Vc in plasma following *po* of Vc is smaller than that in the control group.

The supplement of vitamin-C tablets to the glaucoma patients or to the cataract patients undergoing ECCE surgery may help in the controlling the IOP.

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口服维生素 C 对人眼房水中抗坏血酸盐浓度的影响

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关键词 眼房水；抗坏血酸；血浆；白内障摘除术；高压液相色谱法；氢醌类

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