Polymorphisms in sodium-dependent vitamin C transporter genes and plasma, aqueous humor and lens nucleus ascorbate concentrations in an ascorbate depleted setting

Srinivasan Senthilkumaria, Badri Talwarb, Kuppamuthu Dharsemblingac, Ravilla D. Ravindranb, Ramamurthy Jayanthib, Periasamy Sundaresand, Charu Saravanand, Ian S. Younge, Alan D. Dangourf, Astrid E. Fletcherf,*

a Department of Ocular Pharmacology, Aravind Medical Research Foundation, Madurai, Tamilnadu, India
b Aravind Eye Hospital Pondicherry, Aravind Eye Care, Pondicherry, India
c Department of Proteomics, Aravind Medical Research Foundation, Madurai, Tamilnadu, India
d Department of Genetics, Aravind Medical Research Foundation, Madurai, Tamilnadu, India
e Centre for Public Health, School of Medicine, Dentistry and Biomedical Sciences, Queen’s University Belfast, Belfast, United Kingdom
f Faculty of Epidemiology & Population Health, London School of Hygiene & Tropical Medicine, Keppel St, London WC1E 7HT, United Kingdom

ARTICLE INFO

Article history:
Received 24 April 2014
Accepted in revised form 28 April 2014
Available online 8 May 2014

Keywords:
plasma ascorbate
d lens nucleus ascorbate
aqueous humor ascorbate
cataract
SLC23A1
SLC23A2

ABSTRACT

We have previously reported low concentrations of plasma ascorbate and low dietary vitamin C intake in the older Indian population and a strong inverse association of these with cataract. Little is known about ascorbate levels in aqueous humor and lens in populations habitually depleted of ascorbate and no studies in any setting have investigated whether genetic polymorphisms influence ascorbate levels in ocular tissues. Our objectives were to investigate relationships between ascorbate concentrations in plasma, aqueous humor and lens and whether these relationships are influenced by Single Nucleotide Polymorphisms (SNPs) in sodium-dependent vitamin C transporter genes (SLC23A1 and SLC23A2). We enrolled sixty patients (equal numbers of men and women, mean age 63 years) undergoing small incision cataract surgery in southern India. We measured ascorbate concentrations in plasma, aqueous humor and lens nucleus using high performance liquid chromatography. SLC23A1 SNPs (rs4257763, rs6596473) and SLC23A2 SNPs (rs1279683 and rs12479919) were genotyped using a TaqMan assay. Patients were interviewed for lifestyle factors which might influence ascorbate. Plasma vitamin C was normalized by a log10 transformation. Statistical analysis used linear regression with the slope of the within-subject associations estimated using beta (β) coefficients. The ascorbate concentrations (μmol/L) were: plasma ascorbate, median and inter-quartile range (IQR), 15.2 (7.8, 34.5), mean (SD) of aqueous humor ascorbate, 1074 (545) and lens nucleus ascorbate, 0.42 (0.16) (μmol/g lens nucleus wet weight).

Minimum allele frequencies were: rs1279683 (0.28), rs12479919 (0.30), rs659647 (0.48). Decreasing concentrations of ocular ascorbate from the common to the rare genotype were observed for rs6596473 and rs12479919. The per allele difference in aqueous humor ascorbate for rs6596473 was /C0217μmol/L, p < 0.04 and a per allele difference in lens nucleus ascorbate of /C00.085μmol/L, p < 0.02 for rs12479919.

The β coefficients for the regression of log10 plasma ascorbate on aqueous humor ascorbate for the GG genotype of rs6596473: GG, β = 1460 compared to carriage of the C allele, CG, β = 1059, CC, β = 1132, p interaction = 0.1. In conclusion we found that compared to studies in well-nourished populations, ascorbate concentrations in the plasma, aqueous humor and lens nucleus were low. We present novel findings that polymorphisms in SLC23A1/2 genes influenced ascorbate concentration in aqueous humor and lens nucleus.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

High concentrations of ascorbate in the ocular tissues (aqueous humor, vitreous and lens) compared to blood and other

* Corresponding author. Tel.: +44 (0)20 7927 2253.
E-mail address: astrid.fletcher@lshtm.ac.uk (A.E. Fletcher).

http://dx.doi.org/10.1016/j.exer.2014.04.022
0014-4835 © 2014 Elsevier Ltd. All rights reserved.
tissues have been documented for well over fifty years in mammalian species including humans (Birch et al., 1933; Reiss et al., 1986; Garland, 1991). In contrast there is a paucity of studies which have investigated the within-subject relationships between ascorbate concentrations in human plasma, lens and aqueous humor (Taylor et al., 1991, 1997; Haung et al., 1997; Taylor et al., 1997; Badhu et al., 2007). These studies are important because they enhance our interpretation of epidemiological studies which, of practical necessity, rely on plasma as the biomarker of ascorbate status when investigating risk factors for cataract whereas the more relevant biomarkers might be ascorbate measured in the ocular tissues. This is particularly applicable to India where cataract is higher than in other populations and starts at an earlier age (Minassian et al., 1990; Nirmalan et al., 2003; Vashist et al., 2011).

The seminal study by Taylor in 1997 was a novel investigation conducted in the US setting characterized by habitually high dietary intake of vitamin C and high levels of plasma ascorbate (Taylor et al., 1997). The study showed a log linear relationship between plasma ascorbate and aqueous humor ascorbate. In contrast the relationship between ascorbate concentrations in the plasma and lens was linear and weaker. It is not known whether similar relationships to those which were obtained in a high vitamin C intake setting would be observed in settings with habitually low vitamin C intakes.

In a large population based study in older people in two locations in north and south India (the INDEYE study) we found a robust inverse association between the prevalence of cataract and vitamin C measured both in plasma and in the diet (Ravindran et al., 2011a). A striking finding from the study was the low dietary intake of vitamin C and the low concentration of plasma ascorbate. Vitamin C deficiency (defined as plasma ascorbate < 11 μmol/L) occurred in a substantial proportion, 73% in north India and 46% in south India (Ravindran et al., 2011b). The INDEYE study had no measures of ascorbate in the aqueous humor or lens and therefore we could not exclude the possibility that, although ascorbate concentrations in plasma were low, ascorbate concentrations in aqueous humor and lens might be proportionately higher and similar to concentrations in well-nourished populations, possibly reflecting increased upregulation by ascorbate transporters in deficient states. A few studies conducted over 40 years ago suggested that aqueous humor levels in India were lower than in western populations but these studies were small, lacked information on lens ascorbate and used outdated methods to estimate ascorbate (Consul et al., 1968). No studies, either in India or elsewhere, have investigated whether ascorbate levels in the ocular tissues are influenced by genetic variation in the sodium-dependent vitamin C transporter (SVCT) proteins, SVCT1 and SVCT2 (Tsukaguchi et al., 1999), coded for by the genes SLC23A1 and SLC23A2 respectively (Eck et al., 2004). SVCT1 is expressed primarily in those tissues responsible for the absorption (small intestine and liver) and reabsorption (kidney) of ascorbate (Tsukaguchi et al., 1999; Wang et al., 2000). SVCT2 is expressed in nearly all cell types including the ciliary, corneal (Tsukaguchi et al., 1999; Talluri et al., 2006) and lens epithelium (Kannan et al., 2001). Currently there is little evidence from studies in humans as to whether ascorbate levels in tissues other than plasma are influenced by variants in SLC23A1 or SLC23A2 and no evidence for the ocular tissues. Therefore we conducted a study in south India to investigate the relationships between concentrations of ascorbate in the plasma, the aqueous humor and the lens and whether these were modified by variants (Single Nucleotide Polymorphisms, SNPs) in the genes encoding SVCT (SLC23A1 and SLC23A2).

2. Materials and methods

2.1. Subjects

A purposive sample of 60 patients was taken to ensure equal numbers of men and women stratified by age, 40–59, 60–69 and 70+. Patients aged 40 and above scheduled for small incision cataract surgery (SICS) at Aravind Eye Hospital, Pondicherry, were considered for the study. Patients with inherited cataract or trauma-related cataract, prior phacoemulsification surgery, acute infection, uveitis, glaucoma, diabetic retinopathy and with current diagnosis of cardiac diseases, renal failure, hyperlipidemia, hepatic impairment and malignancy were excluded from the study.

2.2. Ethics

The study protocol was approved by the Institutional Human Ethics Committee of Aravind Eye Hospital, Madurai. The study participants were recruited after obtaining their full written informed consent. Illiterate subjects had the information leaflet read out to them and provided a thumb impression. The study complied with the guidelines in the Declaration of Helsinki.

2.3. Clinical examination

On the day of admission (day prior to surgery), patients underwent a brief examination including lens photography, measurement of height, weight and mid upper arm circumference (as a measure of overall nutritional status) and were interviewed for use of tobacco and alcohol, factors we have shown previously influence plasma ascorbate in the INDEYE study (Ravindran et al., 2011b). After pupillary dilation digital slit beam images of the lens were taken using the Topcon SL-D7 Digital photo slit lamp for nuclear opacities (Topcon, Tokyo, Japan). Retroillumination images of the lens were taken using the Neitz CT-S Cataract Screener for cortical and posterior sub-capsular opacities (PSC) (Neitz Instruments Ltd., Tokyo, Japan). Lens images were assigned a decimal grade in 0.1 unit steps up to a maximum of 6.9 according to the Lens Opacities Classification System III (LOCS III) (Chylack et al., 1993) by an experienced grader (BT) who had been trained in the INDEYE study.

2.4. Sample collection

On the following day, an early morning fasting blood sample of 12 mL was collected in a shaded room in three different vacutainer tubes (5 mL clotted blood for serum, 5 mL ethylenediaminetetra-acetic acid (EDTA) unclotted for plasma and 2 mL in sodium fluoride for measuring fasting blood glucose level). The 5 mL blood sample was kept at room temperature for 1 h to allow for adequate clotting. The unclotted blood sample was kept in the refrigerator. Within 2 h of collection, both clotted and unclotted samples were centrifuged at 3000 rpm at 4 °C (using a cold centrifuge) for 15 min and aliquoted. After centrifugation (a) 200 μL of plasma were transferred to each storage tube using a graduated pipette and exactly 400 μL of ice-cold 10% metaphosphoric acid (MPA) was added to each tube and transferred to a −70 °C freezer after a brief mixing, (b) the buffy coat was transferred to a 2 mL storage tube and stored in a −70 °C freezer for future DNA extraction. Fresh MPA solution was made up every two weeks.

2.5. Aqueous humor and lens nucleus

Participants underwent a routine pre-operative examination including pupil dilation using tropicamide 0.8% with phenylephrine hydrochloride 5%. In two hypertensive patients pupillary dilation
was achieved using tropicamide 1% only as phenylephrine is contraindicated in hypertension. During surgery, prior to the injection of viscoelastic, aqueous humor was obtained through a 27-gauge cannula and collected in a graduated tuberculin syringe. The volume was recorded and transferred to the laboratory in ice. The aqueous humor samples were added with exactly the equal volume of 10% MPA and stored at –70 °C. The intact lens nucleus was removed and placed in a 1.5 mL Nunc vial, transferred immediately to the hospital laboratory, flash frozen in liquid nitrogen and stored at –70 °C. Prior to analysis for ascorbate content, 10 volumes of 10% MPA were added to the pre-weighed lens nucleus, and the resulting solution was homogenized using a polytron homogenizer (Kine- nematica, Japan). The lens nucleus homogenate was centrifuged at 10,000 rpm in a cold centrifuge and the supernatant was analyzed for ascorbate content.

2.6. Ascorbate analysis

Ascorbate concentrations in the plasma, aqueous humor and lens nucleus were measured using a Shimadzu Prominence high performance liquid chromatography (HPLC) system with Photo- diode Array detector (PDA) (Shimadzu Corporation, Kyoto, Japan) in the Department of Ocular Pharmacology, Aravind Medical Research Foundation (AMRF). The analytical separation was achieved with a mobile phase consisting of 0.2 M potassium di hydrogen phosphate containing 2 mM EDTA buffer (pH3) and methanol (95:5 v/v) pumped at the flow rate of 0.6 mL/min into a Luna C18 (250 × 4.6 mm; 5 μm; Phenomenex, California, USA) column. The quantification of ascorbate was carried at UV 245 nm, and its spectral matching was confirmed by an in-built library matching facility. The limit of detection of the assay of ascorbate by HPLC was 1 μmol/L.

2.7. Genotyping

Genomic DNA was extracted from peripheral blood leukocytes in the buffy coat using Quiagen kits. We used a TaqMan assay in an ABI (Applied Biosystems) 7900 HT Fast Real-Time PCR system to genotype two SNPs in the sodium transporter gene SLC23A1 on chromosome 5, rs4257763, Intron 10 and rs6596473, Intron 13) and two SNPs in the sodium transporter gene SLC23A2 on chromosome 20 (rs1279683, Intron 1 and rs12479919, Intron 2). We selected SNPs with evidence from previous studies in humans of an association with plasma ascorbate as a starting point to investigate the association in other tissues (Cahill et al., 2009; Timpson et al., 2010; Zanon-Moreno et al., 2011). Of these, SNPs (e.g. rs339723313) with low Minimum Allele Frequencies (MAF) (<0.1) were excluded as our study did not have power to investigate these.

2.8. Statistical methods

Statistical analysis was carried out using Stata 11 (StataCorp. 2009. Stata Statistical Software: Release 11. College Station, TX: StataCorp LP). We used linear regression to investigate the within- subject relationship between plasma ascorbate and aqueous humor (Fig. 1) or lens nucleus ascorbate with adjustment for confounders. Since the plasma ascorbate concentration was right skewed and not normally distributed (p < 0.00002, Shapiro–Wilk test for departure from normality) we used a log10 transformation which normalized the distribution. To aid interpretation of the results we calculated the increase in aqueous humor for a 10% increase in the log10 plasma ascorbate from the regression equation \( \beta \log_{10}(1.10) \) where \( \beta \) is the coefficient of log10 plasma ascorbate in the model. Lens nucleus and aqueous humor ascorbate were normally distributed. We checked all models by plots of the residuals. We investigated whether individual SNPs modified the coefficients by including a term for the interaction of log10 plasma ascorbate with SNP genotype and used “lincom” commands for the estimates of the coefficients by genotype. We calculated the sample size based on the results from the Taylor study which reported a slope of 1.6 in the regression of log10 plasma ascorbate (mM) against aqueous humor ascorbate (mM) and a standard deviation of 0.58 (Taylor et al., 1997). Assuming a correlation of 0.4 between plasma and aqueous humor ascorbate, a power of 0.9 and an alpha of 0.05, 57 patients were required to detect a similar slope of 1.6 (Dupont et al., 1998).

3. Results

Sixty patients were recruited (30 men and 30 women). No patient refused participation in the study. Nuclear cataract (LOCS III ≥4) was the most common cataract, observed in 48 people; of these 29 had only nuclear cataract, 18 had nuclear and posterior subcapsular cataracts (PSC), and one person had nuclear, PSC and cortical cataracts. The mean nuclear cataract score of all patients was 4.5 (SD = 0.5). The mean age of patients was 63 years, 53% classified themselves as laborers, 78% of women and 21% of men were illiterate (Table 1). Tobacco use was common with 43% reporting any use of tobacco; the pattern differed by sex with chewing tobacco more common in women and smoking tobacco more common in men. Only men reported using alcohol regularly. The mean BMI was 21 kg/m² in both men and women; 6 men and 7

Table 1

<table>
<thead>
<tr>
<th>Characteristics of patients by sex.</th>
<th>Men n = 30</th>
<th>Women n = 30</th>
<th>All n = 60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (^a) (Mean (standard deviation))</td>
<td>63.1 (8.9)</td>
<td>63.8 (8.7)</td>
<td>63.4 (8.7)</td>
</tr>
<tr>
<td>Illiterate (%)</td>
<td>6 (21.4)</td>
<td>22 (78.3)</td>
<td>28 (46.7)</td>
</tr>
<tr>
<td>Occupation (%)</td>
<td>32 (53.3)</td>
<td>32 (53.3)</td>
<td>32 (53.3)</td>
</tr>
<tr>
<td>Laborer</td>
<td>21 (65.6)</td>
<td>11 (36.7)</td>
<td>32 (53.3)</td>
</tr>
<tr>
<td>Retired/unemployed</td>
<td>7 (23.3)</td>
<td>6 (20.0)</td>
<td>13 (21.7)</td>
</tr>
<tr>
<td>Housework/other</td>
<td>2 (0.1)</td>
<td>13 (43.3)</td>
<td>15 (25.0)</td>
</tr>
<tr>
<td>Any Tobacco use (%)</td>
<td>15 (57.7)</td>
<td>11 (42.3)</td>
<td>26 (43.3)</td>
</tr>
<tr>
<td>Chew tobacco</td>
<td>5 (16.7)</td>
<td>11 (36.7)</td>
<td>16 (26.7)</td>
</tr>
<tr>
<td>Smoke tobacco</td>
<td>12 (40.0)</td>
<td>0</td>
<td>12 (20)</td>
</tr>
<tr>
<td>Regular alcohol use (%)</td>
<td>19 (63.3)</td>
<td>0</td>
<td>19 (31.7)</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>21.2 (3.8)</td>
<td>21.7 (3.6)</td>
<td>21.5 (3.7)</td>
</tr>
</tbody>
</table>

\(^a\) Mean (standard deviation).
women were overweight (BMI \geq 25 kg/m²) but none were obese (BMI \geq 30 kg/m²). No patients were taking supplements. The mean (SD) weight of the lens was 154 mg (33), range 100 mg–280 mg. In one patient, lens nucleus ascorbate measurement was not possible due to a hypermature cataract but the results for plasma and aqueous humor were included.

The ascorbate concentrations (\mu mol/L) were: plasma ascorbate, median and inter-quartile range (IQR), 15.2 (7.8, 34.5) and mean (SD) of aqueous humor ascorbate, 1074 (545) and lens nucleus ascorbate, 0.42 (0.16) (\mu mol/g lens nucleus wet weight) (Table 2). Nearly 40% (23/60) of the sample were classified as ascorbate deficient based on their plasma ascorbate concentrations (<11 \mu mol/L). There were no significant differences between men and women in any ascorbate measurements. Age was independently inversely associated with aqueous humor ascorbate (p < 0.0001) and lens nucleus ascorbate (p = 0.03) but not with log₁₀ plasma ascorbate. Increasing nuclear opacity score was associated with lower concentration of lens nucleus ascorbate (p = 0.03), but not with plasma ascorbate (p = 0.9) or aqueous humor ascorbate (p = 0.3). Within-subject regression analyses of log₁₀ plasma ascorbate and aqueous humor ascorbate were therefore adjusted for the effects of age and, additionally in analyses of lens nucleus ascorbate, for nuclear opacity score. No other covariates were confounders in the regression models. We excluded one outlier from the within subject analyses of log₁₀ plasma ascorbate and aqueous humor ascorbate. There was no pattern in the residual plots of log₁₀ plasma ascorbate with either aqueous humor ascorbate or lens nucleus ascorbate.

There was a strong positive association between log₁₀ plasma ascorbate concentration and aqueous humor ascorbate concentration, \beta coefficient = 1156, 95% confidence interval (CI) (948, 1364), p = 10⁻²⁸ (Fig. 2), interpreted as a 48% increase in aqueous humor ascorbate with a 10% increase in log₁₀ plasma ascorbate. Log₁₀ plasma ascorbate concentration was also positively associated with lens nucleus ascorbate concentration, \beta = 0.26, 95% CI (0.17, 0.35), p = 10⁻⁸ (Fig. 3). The relationship between aqueous humor ascorbate and lens nucleus ascorbate concentration was linear, \beta = 0.16 \times 10⁻³, 95% CI (0.10 \times 10⁻³, 0.23 \times 10⁻³), p = 10⁻⁵ (Fig. 4).

The SNPs in SLC23A1 (rs6596473 and rs4257763) were highly correlated with a Linkage Disequilibrium (LD) of 0.90 (r²), and we show the results only for rs6596473. The Minimum Allele Frequency (MAF) was 0.48 for rs6596473, 0.25 for rs12479919 and 0.22 for rs12799683. In the absence of any published data on these SNPs in India, we compared the genotype frequencies with those published in HapMap (http://www.ncbi.nlm.nih.gov/projects/SNP/) either for Gujarati Indians in Houston, Texas, (GIH) when available: rs6596473 (MAF = 0.47), rs12799683 (MAF = 0.28) or across all studies when GIH results were not available, rs12479919 (MAF = 0.30). All three SNPs were in Hardy Weinberg Equilibrium (HWE).

Table 3 shows decreasing concentrations from the common to the rare genotype for aqueous humor ascorbate and rs6596473, per allele difference of –217 \mu mol/L, p < 0.04 and for rs12479919 with lens nucleus ascorbate, per allele difference of –0.85 \mu mol/g, p < 0.02. In models including all three SNPs these differences were not materially attenuated. The coefficients for the regression of log₁₀ plasma ascorbate on aqueous humor ascorbate were higher for the GG genotype of rs6596473: GG, \beta = 1460 compared to carriage of the C allele, CG, \beta = 1059, CC, \beta = 1132, p interaction = 0.1. Results for regression of log₁₀ plasma ascorbate with lens nucleus ascorbate were: GG, \beta = 0.33, CG, \beta = 0.25, CC, \beta = 0.23, p interaction = 0.4. Estimates of effect modification were unreliable for rs12479919 and rs12799683 due to the small numbers in the rare homozygous genotype.

4. Discussion

We present novel data on the relationships between ascorbate concentrations in plasma, aqueous humor and lens nucleus in cataract patients from a population with habitually low vitamin C intake and show that variants in sodium-dependent vitamin C transporter genes influence concentrations in ocular tissues. We did not measure dietary intakes in the present study because of the difficulty of the collection and standardization of measurement of diet in the clinic setting. In our previous study (Ravindran et al.,...
Where we conducted 24 h diet recall, we found very low intakes of dietary vitamin C. In 3000 people aged 60 and over, randomly sampled from villages and small towns in the catchment area of Aravind Eye Hospital, Pondicherry (the same hospital as the present study), the median dietary vitamin C intake was 35 mg/day and plasma ascorbate concentration was 12.9 μmol/L, similar to that observed in the present study. Low dietary intakes have been observed in national surveys in India, of which the most recent reported median intakes of 30 mg/day in men and 23 mg/day similar to women (National Institute of Nutrition, 2006). In contrast, in the US National Health and Examination Survey (NHANES), mean intakes of dietary vitamin C were 96 mg/day, increasing to 208 mg/day in the 48% of the population who also took vitamin supplements (National Institute of Nutrition, 2006). In the US and European populations, median plasma ascorbate concentrations were 37 (SD = 11) μmol/L and 48 (SD = 17) μmol/L, but the aqueous humor concentrations were lower at 993 (SD = 256) μmol/L and 1334 (SD = 494) μmol/L (Badhu et al., 2007) and more comparable to our study.

Few studies have investigated lens ascorbate concentrations in humans (Taylor et al., 1991, 1997; Tessier et al., 1998). Comparison of results across studies is limited by differences in the surgical technique of lens extraction and in the units for expressing lens ascorbate. In two US studies conducted by Taylor et al. some 15 and 20 years ago in patients undergoing phacoemulsification (Taylor et al., 1991, 1997), the results for lens ascorbate was estimated on the assumption that lens volume in all patients was 0.25 mL. This approach permitted a direct comparison of lens ascorbate with plasma and aqueous humor ascorbate since all measures were expressed in the same units. We used the concentration per lens nucleus weight in grams, and measured ascorbate only in the lens nucleus since our method of cataract surgery was SICS, a method in which a portion of the cortex is lost during lens removal. Ascorbate concentration in the lens nucleus may differ from those in the cortex, as was demonstrated in an earlier study in rats (Rosen et al., 1938). However no differences were found for lens ascorbate by type of cataract in a study which measured ascorbate in samples of lens cortico-nuclear blocks in patients undergoing extracapsular extraction (Kisic et al., 2012). A study in French cataract patients was comparable to our method of estimating lens concentrations of ascorbate (μmol/g lens weight) in lens nuclei (Tessier et al., 1998). The authors found lens nucleus ascorbate concentrations of 0.80 μmol/g with mild cataract and 0.50 with severe cataract compared to lens nucleus ascorbate concentrations of 0.42 μmol/g in our study with a mean nuclear LOC5 score of 4.5. We also found decreasing lens nucleus ascorbate with increasing cataract severity but full interpretation of the two studies is limited by differences in the methods of cataract grading.

The slope between plasma and aqueous humor ascorbate in our study was shallower than that reported by Taylor et al. (1997). Using milliMolar (mM) units for comparison, the coefficient in our study was 1.16 mM aqueous humor ascorbate/\log_{10} 10 mM plasma ascorbate, 95% CI (0.95, 1.36) compared to 1.6 mM aqueous humor ascorbate/\log_{10} 10 mM plasma ascorbate in that study. In our study, for every 10% increase in plasma ascorbate there was a 48% increase in aqueous humor ascorbate compared to a 66% increase in the US study. When stratified by the SLC23A1 SNP rs659647, the β coefficient for the common homozygous genotype (GG) was 1.5 mM and closer to that reported in the US study while the β coefficient for the C allele was lower (β = 1.1 mM).

The two genes encoding SVCT1 (SLC23A1) and SVCT2 (SLC23A2) differ considerably in their size and number of SNPs. SLC23A1 on chromosome 5 spans 16 kilobytes while SLC23A2 on chromosome 20 is 10 times larger (Buzrze et al., 2013). Around 200 SNPs have been identified in SLC23A1 and over 10 times as many in SLC23A2 (Michels et al., 2013). Few data are available on population allele frequencies in any ancestral group in either gene and little is known about the functional significance of most SNPs. Studies investigating four exon located SNPs in SLC23A1 reported reduced ascorbate transport in Xenopus oocytes (Corpe et al., 2010) or lower plasma ascorbate levels in humans (Timpson et al., 2010; Duell et al., 2013). The MAFs for these SNPs were less than 5% suggesting the rare allele was associated with unfavorable survival. Of these exon SNPs in SLC23A2, none were associated with the various conditions and diseases studied (Erichsen et al., 2006, 2008;
Dalgard et al., 2013). In both genes SNPs from introns without known function have been associated with plasma ascorbate, in SLC23A1 (Cahill et al., 2009; Timpson et al., 2010; Duell et al., 2013) and SLC23A2 (Zanon-Moreno et al., 2011; Duell et al., 2013) and for various diseases or conditions (Erichsen et al., 2006, 2008; Sikblod et al., 2008; Andrew et al., 2009; Wright et al., 2009; Zanon-Moreno et al., 2011; Dalgard et al., 2013; Duell et al., 2013).

In our study we investigated a common SLC23A1 SNP (rs6596473) located in the Intron region. We found a higher MAF in Indians (0.48 our study and 0.47 in HapMap Gujarati Indians) than HapMap European ancestry populations (0.29), (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=6596473). Population based studies have also reported MAFs of around 0.3 for rs6596473 in European ancestry and per allele differences of between 1 and 3 μmol/L in serum ascorbate (Cahill et al., 2009; Timpson et al., 2010), similar to the observed per allele differences in plasma ascorbate in our study. Since SLC23A1 encodes SVCT1 it is likely that the differences in aqueous humor concentrations by rs659647 genotype reflect variations in absorption or reabsorption of plasma ascorbate outside the ocular tissues, since there is no evidence that SVCT1 is expressed in the corneal epithelium (Tsukaguchi et al., 1999; Talluri et al., 2006) or in human lens epithelial cells (Kapoor et al., 2008).

MAFs for the two SLC23A2 SNPs we investigated were similar to those shown in HapMap. We found a significant relationship between rs12479919 and lens nucleus ascorbate. Given the known role of SVCT2 in ascorbate transport across the lens epithelium, this suggests that rs12479919 affects SVCT2 function or is in linkage disequilibrium with a functional gene variant. Previous studies have reported an association of rs12479919 and gastric (Wright et al., 2009) and bladder cancer (Andrew et al., 2009) but not with colorectal adenoma (Erichsen et al., 2008) or pre-term membrane rupture (Erichsen et al., 2006). The SLC23A2 SNP rs1279683 showed an association with plasma and with aqueous ascorbate in our study although the evidence was not strong (p = 0.1 and 0.2 respectively). This SNP was associated with plasma ascorbate and increased risk of primary open glaucoma (POAG) (Zanon-Moreno et al., 2011). Plasma ascorbate was lower in the G homozygous genotype compared to the C homozygous and heterozygous genotypes, similar to the results we found although the ascorbate concentrations were higher in that study compared to our participants. A further publication from the group confirmed these findings and additionally reported a gene—gene interaction on POAG risk, between rs1279683 and EC14L2/TA, a gene involved in vitamin E transport (Zanon-Moreno et al., 2013). Interestingly rs12479919 and a variant in the SCARB gene known to influence cholesterol transport and thereby vitamin E transport, showed a strong gene—gene interaction on bladder cancer risk (Andrew et al., 2009). These results may also be relevant to lens protection where ascorbate acts synergistically with vitamin E, and both vitamin C and E maintain the antioxidant activity of glutathione (Shang et al., 2003).

Our study was conducted in participants with low concentrations of plasma ascorbate, of whom 40% met the criteria for ascorbate deficiency. We saw no evidence for a saturation threshold of plasma ascorbate with aqueous or lens nucleus ascorbate in the range of plasma ascorbate in our study, a similar finding to the study by Taylor et al. with higher plasma ascorbate concentrations (Taylor et al., 1997). Currently there is limited evidence regarding the mechanisms and pathways of SVCT regulation especially in deficient states. SVCT1 expression is reduced with ascorbate supplementation and increased with ascorbate depletion (Lindblad et al., 2013). SVCT2 regulation is influenced by organ or tissue specific intracellular ascorbate concentration and tissue specific functional requirements, for example SVCT2 upregulation after exposure to oxidative stress in human lens epithelial cells (Kannan et al., 2001).

Some limitations of our study need consideration. We recruited patients undergoing cataract surgery and our results may not apply to the general population. Studies of human ocular tissues inevitably use patients with eye diseases to collect samples, such as lens or aqueous humor during cataract extraction or vitreous humor after vitrectomy. Cadaver studies are not appropriate due to the degradation of ascorbate in body tissues after death.

We did not measure the oxidized form of ascorbate, dehydroascorbate (DHA). There are considerable difficulties in measuring DHA in human subjects. The few studies that have attempted this in plasma or serum have estimated DHA indirectly from the difference between total ascorbate and reduced ascorbate (subtraction method) which requires treating the sample with a reductant. This method may be unreliable as the in vivo equilibrium between ascorbate and DHA is not completely blocked leading to inaccurate estimation of DHA (Michels et al., 2013). SVCT transporters have high affinity for ascorbate but not for DHA. DHA is transported by facilitative glucose transporters (GLUT) into cells, including aqueous humor and lens, where it is rapidly reduced to ascorbate (Wilson, 2005). GLUT transporters are expressed in the corneal and in the lens epithelium (Umapathy et al., 2013). The evidence that SVCT, rather than GLUT, is the major influence on ascorbate absorption and accumulation is based on studies showing negligible DHA in plasma compared to ascorbate (Dhariwal et al., 1991) and that knockout mice lacking SVCT2 have almost undetectable ascorbate concentrations and die shortly after birth (Sotiriou et al., 2002). However DHA plays an important role in ascorbate recycling due to the ubiquitous GLUT transporters especially in situations of high oxidative stress and further research using GLUT transporters and optimum methods of DHA measurement are warranted.

We did not investigate variants in other genes which may also influence ascorbate concentrations such as glutathione S-transferase and superoxide dismutase (Michels et al., 2013). Of particular relevance to our setting is a polymorphism in the haptoglobin (Hp) gene since the haptoglobin Hp 2-2 genotype/phenotype occurs in 70%–80% of Indians compared to populations of European ancestry (30–40%) (Carter et al., 2007). The Hp 2-2 phenotype has been reported to reduce plasma vitamin C levels by as much 25% in European populations with adequate dietary intakes of vitamin C (Langlois et al., 1997).

In summary we found that compared to studies in well-nourished populations, ascorbate concentrations in the plasma, aqueous humor and lens nucleus were low. We present novel findings that polymorphisms in SLC23A1/2 genes are associated with ascorbate concentrations in aqueous humor and lens nucleus. Further investigation of genetic influences on ascorbate in ocular tissues in different population settings with variation in allele frequencies and dietary intakes will help to elucidate the role of vitamin C in cataract.

Acknowledgments

Dr Caroline Mercer from Queens University Belfast for input on the methodology of ascorbate measurement.

References

