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Short communication

The effect of aspirin and vitamins C and E on HbA_{1c} assays

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Abstract

Background: Aspirin (ASA) and vitamins C and E may inhibit non-enzymatic glycation in vivo and may also interfere with HbA_{1c} assays, masking true results. We investigated the effect of usual doses of ASA, vitamin C and E on HbA1c levels in a group of non-diabetic volunteers. *Methods:* A randomized clinical trial was performed with 28 healthy non-diabetic individuals. Subjects were allocated to take ASA 200 mg/day, vitamin C 1 g/day, vitamin E 400 mg/day, or to a control group, for a period of 4 months. Blood samples were collected at baseline and at monthly intervals for HbA1c analysis by HPLC Variant II (BioRad), HPLC L-9100 (Merck – Hitachi) and Tina Quant® HbA_{1c} II immunoassay (Roche). *Results:* HbA_{1c} levels of the control, vitamin C and E groups did not change throughout the study, independently of the method used. HbA_{1c} measured by Hitachi L-9100 HPLC increased significantly ($P=0.033$) at 4 months after ASA intake, although this increase was of only 0.17%. *Conclusions:* Treatment with vitamins C and E in pharmacological doses does not have any impact on HbA1c measurements in non-diabetic patients with the three methods employed. ASA induces a modest, not clinically relevant, increase in HbA1c levels with one of the methods.

Keywords: Glycemic control; Glycohemoglobin; Ascorbic acid; Acetyl salicylic acid; Tocopherol

1. Introduction

After 2 landmark studies – the Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS) – glycated haemoglobin (HbA1c) became the reference parameter to evaluate metabolic control in patients with diabetes [1,2]. Ideally, the assay employed to measure HbA1c should be traceable to the DCCT/UKPDS values [3]. Furthermore, the correct interpretation of HbA1c results by physicians requires knowledge of factors that may possibly interfere with HbA1c test results, such as intake of aspirin (acetyl salicylic acid, ASA) and vitamins C and E [4].

ASA is indicated for all patients with diabetes above >30 years and without any contra-indications to decrease the risk of myocardial infarction [5]. ASA promotes acetylation of HbA1c chains, altering the net protein charge. This acetylated product may comigrate with the A_{1c} fraction in assays that are based on charge separation, such as ion exchange chromatography, and it also inhibits in vivo non-enzymatic protein glycation through a site competition mechanism [4]. Both these effects could affect HbA_{1c} results in opposite directions.

Although the use of vitamins C and E might have a protective role in the development of diabetic microvascular complications [6], recent clinical trial did not observe any effect of these vitamins on cardiovascular outcomes and nephropathy in patients with and without diabetes [7]. Vitamins C and E have been reported to decrease protein glycation [8,9], although some reports do not confirm these findings [10,11]. In addition, cross-sectional studies have shown a negative association between the intake of vitamin C and/or E and levels of HbA1c [12].

Therefore the aim of this study was to investigate the effect ASA and of vitamins C and E on HbA1c levels in a group of non-diabetic volunteers.

2. Materials and methods

2.1. Study design

This study followed a randomized control trial design. Subjects were randomized and allocated to take ASA (Aspirin,

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Bayer® SA) 200 mg/day, vitamin C (Cewin®, Sanofi Synthelabo Ltda) 1 g/day, vitamin E (Ephynal®, acetate capsules 400 mg, Roche® Pharmaceutical Products) 400 mg/day or to be part of a control group. Blood samples were collected for HbA1c (at baseline and at monthly intervals, for a period of 120 days), basal biochemistry, hematological and drugs analysis. The randomization process was performed according to a computer generated randomization list. The sample size was calculated based on the standard deviation (SD) of the reference interval for HbA1c at our laboratory (0.30%). A minimum of 6 patients in each group was necessary to detect a 0.5% absolute change in HbA1c (α=0.05 and β=0.20) in relation to baseline levels.

2.2. Subjects

Potential participants were excluded if they had contra-indications for ASA use, if they had been regularly taking vitamins C, E and/or multivitamins, and if they presented abnormal hematological status, dyslipidemia (triglycerides >4.4 mmol/l) or renal disease (serum creatinine >133 μmol/l). Thirty-four non-diabetic volunteers (14 men, non-diabetic status confirmed by WHO criteria) were enrolled.

During the study they were instructed to eat normally, refrain to use ASA, vitamin C, E and multivitamins, and to avoid excess alcohol intake (>10 drinks/week). All subjects included in the study were white, and all signed an informed consent form.

2.3. Analytical methods

Glucose, total cholesterol, HDL-cholesterol, triglycerides, and creatinine were analyzed in the same day of blood collection by Advia 1650 (Bayer® Diagnostic). Hematological analysis was performed using a Pentra 2000 Automated System (ABX® Diagnostic System). HbA1c levels were batch measured by HPLC Variant II (BioRad Laboratories, Hercules CA), HPLC L-9100 Glycated Hemoglobin Analyzer (Merck – Hitachi, Tokyo, Japan), and Tina Quant® Hemoglobin A_{1c} II immunoassay (Roche Diagnostics, Mannheim). Serum vitamin C and E were batch analysed by HPLC. Serum ASA levels were measured in batches by dry chemistry (Vitros ECi, Ortho Clinical Diagnostics, Rochester, NY).

2.4. Statistical analysis

ANOVA with Tukey correction was used to compare HbA_{1c} results throughout the treatment in the different groups and Mann Whitney test was used to compare the drugs levels at baseline and after four months treatment, at a significance level of 0.05. Within-subject variation was calculated as the coefficient of variation (CV) of HbA_{1c} results for the same individual, measured by the same method, throughout the study.

2.5. Ethical aspects

The study protocol was approved by the Ethics Committee of Hospital de Clinicas de Porto Alegre.

3. Results

Twenty-eight subjects completed the study. Six subjects (3 men) interrupted the treatment before the end of the third month (2 in the vitamin E group; 3 in the vitamin C group; and 1 in the ASA group). Five individuals dropped out due to non-compliance (Vitamin C and E groups) and one person from the ASA group presented with gastric discomfort and stopped treatment. Table 1 shows the baseline characteristics of 28 non-diabetic volunteers that completed the study. There were no differences among the 4 groups concerning age, sex, and glucose and lipids levels at baseline.

In patients taking vitamin C or E, there was a significant increase in serum vitamin levels after 4 months in relation to baseline levels and to the control group. Serum vitamin C levels increased by 38.5% [39.75 μmol/l (34.07–45.42) vs. 51.10 μmol/l (39.75–62.46); P=0.0374]. Vitamin E levels increased by 80.0% [17.81 μmol/l (2.03–25.44) vs. 26.46 μmol/l (12.46–47.06); P=0.0327]. There was no significant difference on serum levels of salicylate in the ASA group after 4 months of treatment.

For each method, baseline HbA_{1c} levels were similar for all groups (Table 2). HbA_{1c} levels obtained using the Tina Quant II

Table 1
Baseline characteristics of 28 non-diabetic volunteers

	Controls	Vitamin C	Vitamin E	ASA
N	7	7	7	7
Sex (F/M)	4/3	5/2	5/2	3/4
Age (years)	27±7	30±8	29±8	30±12
Fasting glucose (mmol/l)	4.70±0.51	4.40±0.65	4.97±0.72	4.75±0.40
Glucose 2 h after load (mmol/l)	4.80±0.87	5.30±1.31	5.08±1.27	5.00±1.12
Total hemoglobin (g/l)	137±14	138±15	128±11	148±11
Total cholesterol (mmol/l)	4.55±0.64	5.10±0.79	4.71±1.16	5.15±0.55
HDL-cholesterol (mmol/l)	1.56±0.28	1.38±0.33	1.48±0.36	1.46±0.15
Triglycerides (mmol/l)	0.88±0.43	1.53±0.97	0.75±0.24	0.97±0.56
Serum vitamin C (μmol/l)	39.75 (34.07–45.42)	51.10 (39.75–62.46)	–	–
Serum vitamin E (μmol/l)	17.81 (2.03–25.44)	–	26.46 (12.46–47.06)	–
Serum ASA (mg/l, range)	<1.0–2.0	–	–	<1.0–2.0

Table 2
HbA_{1c} levels in non-diabetic volunteers before, during and after vitamin C, vitamin E and aspirin (ASA) treatment for 4 months

HbA_{1c} (%)		Baseline	30 days	60 days	90 days	120 days
Controls	HPLC 1	4.63 (±0.24)	4.70 (±0.46)	4.87 (±0.20)	4.80 (±0.24)	4.67 (±0.30)
	HPLC 2	4.70 (±0.24)	4.62 (±0.24)	4.70 (±0.27)	4.70 (±0.29)	4.68 (±0.23)
	IA	5.20 (±0.39)	5.18 (±0.29)	5.15 (±0.21)	5.14 (±0.17)	5.07 (±0.23)
Vitamin C*	HPLC 1	4.73 (±0.44)	4.57 (±0.26)	4.78 (±0.26)	4.83 (±0.28)	4.91 (±0.29)
	HPLC 2	4.87 (±0.17)	4.80 (±0.25)	4.77 (±0.25)	4.78 (±0.25)	4.87 (±0.24)
	IA	5.29 (±0.26)	5.27 (±0.29)	5.10 (±0.16)	5.17 (±0.34)	5.19 (±0.41)
Vitamin E†	HPLC 1	4.61 (±0.20)	4.74 (±0.21)	4.96 (±0.27)	4.90 (±0.25)	4.73 (±0.34)
	HPLC 2	4.94 (±0.14)	4.92 (±0.23)	5.01 (±0.27)	5.04 (±0.33)	5.06 (±0.25)
	IA	5.44 (±0.44)	5.53 (±0.47)	5.44 (±0.67)	5.35 (±0.29)	5.48 (±0.54)
ASA‡	HPLC 1	4.57 (±0.17)	4.60 (±0.17)	4.80 (±0.39)	4.65 (±0.23)	4.74 (±0.21)
	HPLC 2§	4.84 (±0.24)	4.74 (±0.17)	4.77 (±0.14)	4.93 (±0.06)	4.94 (±0.10)
	IA	5.14 (±0.30)	5.10 (±0.30)	5.13 (±0.32)	5.13 (±0.32)	5.33 (±0.40)

*Serum levels = 39.75 μmol/l (34.07–44.42 at baseline vs. 51.10 μmol/l (39.75–62.46) at 120 days, $P = 0.0374$; †serum levels = 17.81 μmol/l (2.03–25.44) at baseline vs. 26.46 μmol/l (12.46–47.06) at 120 days, $P = 0.0327$; ‡serum levels ranged from <1 to 2 mg/l, $P > 0.05$; §$P = 0.033$ for 30 vs. 120 days. HPLC 1 – Variant II BioRad; HPLC 2 – Hitachi L9100; IA – immunoassay Tina Quant II. Results are expressed as mean (±SD) for HbA_{1c} and as median (range) for vitamins C and E.

immunoassay were significantly higher than those obtained using the Variant II HPLC system ($P < 0.001$). Inter-assay CVs were 2.5%, 3.89% and 4.30% for Hitachi L-9100 HPLC, BioRad Variant II HPLC and Tina Quant® II immunoassay, respectively.

The HbA_{1c} levels of the control, vitamin C and E groups did not change throughout the study, independently of the method used for measurement. A significant difference was observed between HbA_{1c} levels at 30 and 120 days of ASA treatment with the Hitachi L-9100 HPLC assay. Although significant ($P = 0.033$), this difference was very small (0.19%). The other assays did not reveal any significant changes in HbA_{1c} results after ASA treatment. Within-subject variation of HbA_{1c} levels, measured by the same method, was small and similar for the different groups throughout the study (<5%).

4. Discussion

This randomized clinical trial showed that treatment with vitamin C or E in pharmacological doses does not significantly influence HbA_{1c} measurements in non-diabetic patients using the 3 methods studied. Although 200 mg/day of ASA induced a modest increase in HbA_{1c} levels measured by Hitachi L-9100 HPLC, this increase was not detected by either Variant II HPLC or Tina Quant II immunoassay. Neither the HPLC methods (Hitachi L-9100, Variant II) nor the Tina Quant II immunoassay were affected by any hemoglobin derivatives formed during the chronic ingestion of vitamin C and E.

Two of the methods employed in the present study are widely used: 24.9% of the laboratories participating in the College of American Pathologist GH2-B 2005 Survey [13] use the Variant II HPLC and the Tina Quant HbA1c II immunoassay for HbA1c analysis.

The results from previous studies are contradictory concerning the effect of vitamin C on HbA_{1c} measurements and method dependent. Vitamin C did not affect HbA1c determinations by HPLC, electrophoresis, affinity chromatography and immunoassay in a similar study [10], but was reported to greatly affect HbA_{1c} levels when affinity and electrophoresis assays were used in another investigation [8].

Similarly, the effect of vitamin E on HbA_{1c} assays is not well established. There are several clinical and observational studies reporting the effects of vitamin E levels, after supplementation or not, on protein glycation. They consistently show that there is an inverse association between HbA_{1c} and vitamin E levels [9,12]. However, some studies show that the effect of vitamin E was negligible on glycemic control assessed by HbA_{1c} levels, but was protective for renal disease and lipid oxidation [6,11]. These data suggest that the protective effect of vitamin E may result from other mechanisms rather than glycation inhibition. The HOPE clinical trial did not observed any effect of vitamin E on cardiovascular outcomes and nephropathy in patients with and without diabetes [7].

The effect of ASA on HbA_{1c} results is also unclear [4,14]. Most of the data on this topic originated from in vitro experiments; the available in vivo studies were performed with subjects with rheumatoid arthritis in chronic use of much higher doses of ASA (>1 g/day), or with cardiology patients.

The analysis of within-subject variation revealed only a small variation in HbA1c results (intra individual CV < 5%). However, the observed within-subject variation was slightly higher than that found for normal individuals without any treatment in a different study [15]. The changes in HbA_{1c} levels observed in the present study are probably of a biological nature, rather than the result of interference, and they were expected, considering the inter-assay imprecision of the methods used.

In conclusion, the treatment with vitamin C and E does not have any impact on HbA_{1c} measurements in non-diabetic patients with the method evaluated. ASA may induce a modest, although not clinically relevant, increase in HbA_{1c} levels.

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