Effect of smoking on erythorbic acid pharmacokinetics

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Considerable evidence exists that smoking significantly lowers the concentration of plasma antioxidants. While for most antioxidants this effect appears to result mainly from altered dietary habits, ascorbic acid has recently been shown to be depleted by smoking *per se*. However, the direct cause of ascorbate depletion remains unclear. Erythorbic acid is a stereoisomer of ascorbic acid commonly used as antioxidant in foodstuffs and has the same redox properties as ascorbic acid. We therefore investigated if erythorbic acid could be used as a non-isotopic marker of smoking-induced oxidative stress. In a sample of smokers (*n* 10) and non-smokers (*n* 10), the pharmacokinetics of erythorbic acid were followed after a single oral dose (1 g) and subsequently, the effect of a 2-week ascorbic acid supplementation (0·5 g/d) on erythorbic acid kinetics was studied in a double-blind, placebo-controlled fashion. There were no significant effects of smoking or supplementation on relative bioavailability (difference in area under curve, $AUC_{0-\infty}$) of erythorbic acid (smokers 357 (SD 119), non-smokers 414 (SD 142) μmol·h/l; *P*=0·34). Time to reach maximum plasma concentration (T_{max}) was significantly less in smokers (*P*=0·03). If the relative pharmacokinetics of erythorbic acid between smokers and non-smokers compares with those of AA, our present results do not suggest that altered pharmacokinetics is likely to play a major role in the ascorbic acid depletion consistently observed in smokers.

Erythorbic acid: Ascorbic acid: Smoking: Supplementation: Bioavailability

Smoking has long been associated with several chronic diseases, including cancer and atherosclerosis (Doll *et al*. 1994; Halliwell & Gutteridge, 1999). These observations correlate well with the high number of oxidants and other toxic chemicals contained in tobacco smoke, which relate to modification of cellular macromolecules and consumption of low molecular mass antioxidants (Kiyosawa *et al*. 1990; Frei *et al*. 1991; Leanderson & Tagesson, 1992; Pryor & Stone, 1993; Fraga *et al*. 1996). In addition, poor antioxidant status and increased oxidative damage are consistently observed among smokers (Duthie *et al*. 1991; Loft *et al*. 1992; Duthie & Arthur, 1994; Lykkesfeldt *et al*. 1996, 1997). However, despite the numerous observational studies in which these unfavourable conditions have been demonstrated in smokers, the effect of smoking on plasma antioxidants remains largely unexplained.

We have documented the effect of smoking on the plasma concentration of ascorbic acid (AA; Lykkesfeldt *et al*. 1996, 1997, 2000). However, little is known about the basic pharmacokinetics of AA in smokers *v*. non-smokers, i.e. its relative absorption rate, bioavailability, distribution and excretion. For non-smokers, bioavailability is complex and dose-dependent. Absorption is quantitative during AA deficiency while >500 mg doses are largely excreted or not absorbed (Levine *et al*. 1996).

Erythorbic acid (EA) is an optical isomer of AA that is commonly used in the food industry as antioxidant, although it is not a natural constituent of the human diet. Using EA as a non-isotopic model compound of AA, based on their similar physico-chemical properties, we examined its relative absorption rate, bioavailability and elimination rate following a single oral dose of 1 g EA given to fasting male smokers and non-smokers. Subsequently, to investigate the influence of AA status, the smokers and non-smokers were randomized in a double-blind fashion into subgroups receiving either a 500 mg

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AA supplement/d or placebo for 2 weeks and the EA experiments were repeated.

Methods

Subjects and study design

The study was conducted in accordance with the Declaration of Helsinki and approved by the Local Ethics Committee of Copenhagen. Twenty healthy male volunteers (ten smokers and ten non-smokers) between 20 and 58 years of age were recruited from the greater Copenhagen area using newspaper advertisement. Inclusion criteria were as follows: >18 years of age, smoking more than ten cigarettes per d (smokers only) and signing the informed consent form. Exclusion criteria were chronic diseases, regular use of medicine, any use of vitamin or other dietary supplements and smoking within the last 10 years (non-smokers only). No subjects withdrew or were excluded from the study after recruitment.

Following the collection of an overnight fasting blood sample, each subject ingested a single oral dose of 1 g EA dissolved in 200 ml water and blood samples were taken for the next 8 h at predefined time intervals. In a double-blind fashion, smokers and non-smokers were subsequently each randomized into two groups receiving either a vitamin supplement (500 mg AA/d) or placebo (500 mg NaCl/d having the same visual appearance and taste as the supplement) for 2 weeks. The randomization procedure resulted in uneven *n* values for the four groups (Table 1). Immediately following the supplementation period, the EA experiment was repeated as described earlier.

Collection and analysis of blood samples

Upon arrival in the medical research facility, a peripheral venous catheter was inserted into the *vena cubitalis*. Overnight fasting blood samples were taken into 10 ml vials containing EDTA as anticoagulant. The blood samples were immediately centrifuged at 2000 g for 5 min (4°C), precipitated using metaphosphoric acid as described previously (Lykkesfeldt, 2000, 2002) and stored at −80°C until analysis within 1 month. Blood samples were subsequently taken at 20, 40, 60, 80, 100, 120, 140, 160, 180, 210, 240, 270, 300, 360, 420 and 480 min following the administration of 1 g EA. The concentrations of AA and EA in plasma were measured by using reversed-phase HPLC with coulometric detection as described by Lykkesfeldt (2000, 2002). All standards were from Fluka (Milwaukee, MO, USA).

Statistical analysis

As shown in Table 1, considerable variance was observed for the pharmacokinetic variables. Consequently, it should be emphasized that the conclusions drawn have limited statistical power.

Data were analysed by using Statistica 6 (StatSoft, Tulsa, OK, USA). Homogeneity of variances was verified by Levene's test. Baseline differences between smokers and non-smokers were analysed using one-way ANOVA. Differences post-supplementation were analysed using 2-way ANOVA with smoking and supplementation as factors. A two-tailed *P* value <0.05 was considered statistically significant. Values are reported as means and standard deviations.

Results

Antioxidant measurements

The population sample was initially characterized by measuring plasma concentrations of AA (Table 1) and EA. A 17 % lower AA plasma concentration was found in smokers *v.* non-smokers. The observed differences in baseline AA concentrations did not reach statistical significance, but are comparable in size with those reported previously for larger populations (Lykkesfeldt *et al.* 1996, 1997, 2000). EA was not detected in any of the baseline samples. This suggests that the subjects were indeed fasting as requested. Following a 2-week supplementation period, two-way ANOVA using supplementation and smoking as factor revealed significant effects of both supplementation ($P=0.000033$) and smoking ($P=0.0088$) on plasma AA as expected (Table 1).

Erythorbic acid uptake and elimination

Plasma concentration curves were constructed from a series of blood samples taken after the ingestion of 1 g EA (Fig. 1(A)). Ingestion of EA did not affect the plasma AA concentration (Fig. 1(B)). Relative bioavailability (difference in area under curve, $AUC_{0-\infty}$), half-life ($T_{\frac{1}{2}}$) and maximum plasma concentration (C_{max}) were not significantly different between smokers and non-smokers (Table 1). However, the time to reach the maximum plasma concentration (T_{max}) was significantly less among smokers, indicating a faster uptake of EA ($P=0.03$).

Effects of ascorbic acid supplementation

Supplementation with 500 mg AA for 14 d resulted in markedly increased plasma AA among the supplemented individuals ($P=0.000033$). No significant change was observed in the placebo groups (Table 1). No significant effects were observed on relative EA bioavailability, but the time to reach the maximum plasma concentration (T_{max}) decreased by 15 % in supplemented non-smokers ($P=0.024$).

Fitting to an open two-compartment model

The uptake, distribution and elimination of EA were also analysed by using an open two-compartment pharmacokinetic model (results not shown). Individual data sets were fitted to an appropriate differential equation by using the Statistica (StatSoft) nonlinear estimation module from which the rate constants for absorption, distribution and elimination were calculated. No significant effects of smoking or supplementation were observed.

Table 1. Age and plasma ascorbic acid concentrations for the study population of smokers and non-smokers: pharmacokinetic variables of erythorbic acid are given following a single oral dose (1000 mg) before and after a 2-week supplementation with ascorbic acid (500 mg/d)‡

(Mean values and standard deviations)

	Baseline group statistics				Placebo								Supplement							
	Non-smokers (n 10)		Smokers (n 10)		Non-smokers (n 4)				Smokers (n 6)				Non-smokers (n 6)				Smokers (n 4)			
					Baseline		14 d		Baseline		14 d		Baseline		14 d		Baseline		14 d	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)	29·7	11·1	32·5	10·9	28·5	11·0			29·2	6·2			30·5	12·1			37·5	15·3		
Ascorbic acid (µM)†	53·4	18·6	44·2	17·7	50·0	27·0	46·8	23·1	51·0	15·9	40·0	12·3	55·7	12·9	90	5·2	34·1	17·0	72·8	22·5
Erythorbic acid kinetics																				
$AUC_{0-\infty}$ (µmol·h/l)	414	142	357	119	510	177	446	189	339	120	321	67	350	73	280	118	386	132	312	129
$T_{\frac{1}{2}}$ (min)	200	102	181	44	269	139	211	70	163	15	161	24	153	29	175	63	206	62	139	16
C_{max} (µmol/l)	63·3	13·7	56·0	21·6	66·1	12·8	61·4	21·8	54·3	15·4	53·4	13·3	61·5	15·1	51·9	25·8	58·6	31·4	52·7	20·6
T_{max} (min)*	162	19	142	19	181	14	146	25	142	22	135	15	150	7	125	13	143	18	137	22

$AUC_{0-\infty}$, difference in area/under curve (relative bioavailability); $T_{\frac{1}{2}}$, half-life; C_{max}, maximum plasma concentration; T_{max}, time taken to reach maximum plasma concentration.
* One-way ANOVA showed significant effect of smoking at baseline ($P=0.03$).
† Two-way ANOVA using smoking and supplementation as factors showed $P=0.0088$ for the effect of smoking and $P=0.000033$ for the effect of supplementation.
‡ For details of subjects and procedures, see p. 668.
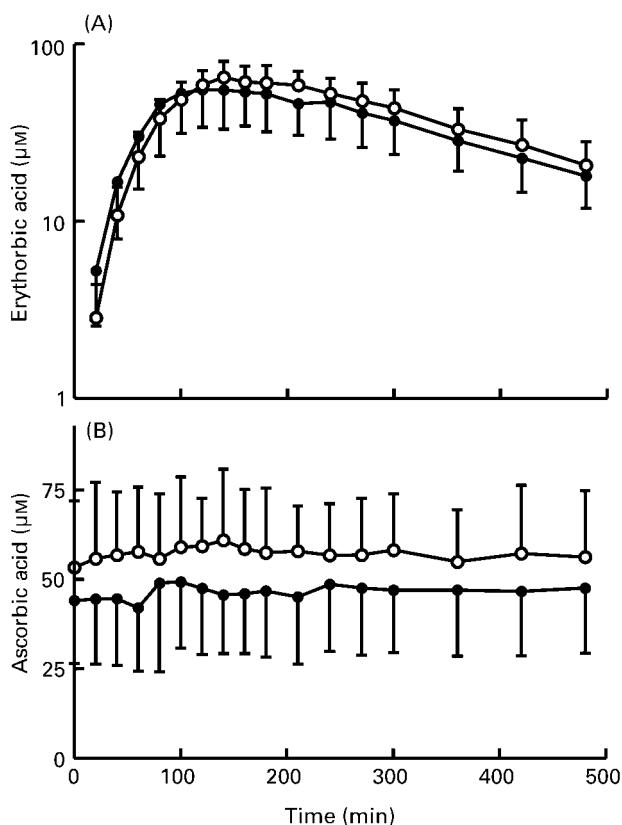
Fig. 1. Plasma concentrations of erythorbic acid ((A), log scale) and ascorbic acid (B), following a single dose of erythorbic acid (1 g). (○), Non-smokers (*n* 10); (●), smokers (*n* 10). For details of subjects and procedures, see Table 1 and p. 668. Values are means for the baseline experiment with standard deviations shown by vertical bars.

Discussion

The effects of smoking on plasma antioxidants have been studied for decades. Specifically, the difference in AA between smokers and non-smokers has been documented by us on several occasions (Lykkesfeldt *et al.* 1996, 1997, 2000). The plasma concentration of AA in human subjects is a function of several variables, e.g. dietary intake, bioavailability, transport, oxidation rate, recycling capacity, catabolism and excretion. Of these variables, differences in dietary intake have been shown to be involved in the AA depletion observed in smokers. However, as shown recently, dietary differences only account for a relatively small proportion of this depletion (Lykkesfeldt *et al.* 2000). Consequently, smoking must also affect one or more of the remaining variables mentioned earlier.

To further elucidate the reason for AA depletion in smokers, we hypothesized a possible involvement of altered pharmacokinetics. However, such studies of an endogenous substance like AA would normally require the use of isotope-labelled AA, which may be ethically questionable. Instead, we used EA as a non-isotopic marker of AA.

EA is a stereoisomer of AA that is commonly used as antioxidant in food products. Consequently, man ingests considerable amounts of EA daily with their regular diet (Sauberlich *et al.* 1991). This constitutes a problem in many assays of AA, which is commonly overestimated, particularly in spectrophotometric assays (Sauberlich *et al.* 1991; Muggli, 1992). In the present study, the method used for AA determination is capable of quantifying both compounds separately (Lykkesfeldt, 2002).

EA cannot replace AA *in vivo*. The anti-scorbutic potency of EA has been estimated to only one-twentieth that of AA (Goldman *et al.* 1981). Moreover, EA pharmacokinetics differs from those of AA at least with respect to elimination and tissue uptake (Hornig & Weiser, 1976; Hornig, 1977; Sauberlich *et al.* 1996). Consequently, only relative effects of smoking can possibly be extrapolated from EA to AA. However, EA has the same redox properties as its biological counterpart AA (Iheanacho *et al.* 1995), and the results of the present study confirmed that their oxidized metabolites, dehydroerythorbic acid and dehydroascorbic acid respectively, are reduced intracellularly with the same efficiency (results not shown). Thus, as smoking apparently results in a considerable consumption of AA *in vivo*, it was conceivable that an effect of smoking could be observed on EA kinetics.

The pharmacokinetics of EA was calculated from the plasma concentration curves shown in Fig. 1(A). Generally, no significant effects of smoking on bioavailability, maximum plasma concentration or elimination were observed. The plasma concentration of AA was not significantly affected by ingestion of EA (Fig. 1(B)). A significantly decreased time to reach the maximum plasma concentration (T_{max}) was observed among smokers, probably due to differences in gastric emptying rate (Scott *et al.* 1993). Further pharmacokinetic analysis based on an open two-compartment model revealed no significant effects of smoking or supplementation on the rate constants of absorption, distribution or elimination (results not shown).

In conclusion, if the relative pharmacokinetics of EA between smokers and non-smokers compares with those of AA, our present results do not suggest that the significantly lower plasma AA in smokers can be explained by decreased bioavailability or increased elimination. However, it should be emphasized that the present study has limited statistical power due to the small number of subjects and that our present results can only be regarded as indicative. Thus, larger studies are needed to confirm the findings of the present work. We believe the results of the present study can pave the road for such investigations.

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