Hyperlipidaemia and the Use of Tocopherol in Antioxidant Cocktails in Smokers

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Abbreviations: ABC study, Alpha-tocopherol Beta-Carotene Cancer Prevention Study; CI, confidence interval; FMD, flow-mediated vasodilation; GPx, glutathione peroxidase; HDL, high-density lipoprotein; IL, interleukin; LDL, low-density lipoprotein; 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; PAI-1, plasminogen activator inhibitor-1; PG, prostaglandin; RR, relative risk; sICAM-1, soluble intercellular adhesion molecule-1; SOD, superoxide dismutase; TBARS, thiobarbituric acid-reactive substances; Tx, thromboxane; VLDL, very low-density lipoprotein.

Abstract

Cigarette smoke, high in oxidants and free radicals, causes lipid peroxidation and oxidative damage. Smokers have imbalanced antioxidant status and greater lipid peroxidation due to increased oxidative stress by cigarette smoke. Lipid peroxidation is the free radical-mediated oxidation of membrane polyunsaturated lipids preceded by a chain reaction that is modulated by pro-oxidants and antioxidants. Tocopherol, the principal lipid-soluble chain-breaking antioxidant in plasma and on the membrane of the tissues, acts as a predominant antioxidant in the low-density lipoprotein particle by trapping peroxyl free radicals. Supplementation with tocopherol alone or in a cocktail may improve antioxidant status and protect smokers from oxidative damage. Current evidence of tocopherol status and supplementation in smokers is illustrated.

Introduction

Cigarette smoke contains two different phases of free radicals, one in the tar and the other in the gas phase (Church and Pryor, 1985). The tar phase contains several relatively stable free radicals, whereas the gas phase contains small oxygen- and carbon-centred radicals which are much more reactive than those in the tar phase. The estimated free radicals are in the order of 10^{14} per inhalation (Church and Pryor, 1985). Because cigarette smoke contains large amounts of xenobiotics, including oxidants and free radicals, it can lead to peroxidation and oxidative damage. Smoking has been reported to cause an increase in oxidative stress and an imbalance in antioxidant nutrient intake and status (Faruque *et al.*, 1995; Mezzetti *et al.*, 1995; Ross *et al.*, 1995; Ma *et al.*, 2000; Wei *et al.*, 2001, Durak *et al.*, 2002). Evidence also further shows that smoking is associated with increased free radical production and antioxidant depletion (Faruque *et al.*, 1995; Polidori *et al.* 2003; Valachovicova *et al.*, 2003). Significantly a decreased plasma antioxidant level, such as that of α-tocopherol, and increased oxidative damage were observed in smokers (Mezzetti *et al.*, 1995; Ross *et al.*, 1995; Durak *et al.*, 2000; Zhou *et al.*, 2000; Wei *et al.*, 2001). Overproduction or impaired neutralization of free radicals accounts for oxidative stress, which can consequently cause damage to endothelial cells of the blood vessels. Long-term smoking is thought to cause endothelial dysfunction through increased oxidative stress (Papamichael *et al.*, 2004). Furthermore, epidemiological data support that smoking is an important risk factor for hyperlipidaemia (Schuitemaker *et al.*, 2002; Wang *et al.*, 2003) (Table 26.1). It is reasonable to assume that antioxidant supplementation may be beneficial to eliminate cellular oxidative damage and alleviate hyperlipidaemia in smokers.

Table 26.1. Definition of free radicals, oxidative stress, endothelial dysfunction and hyperlipidaemia.

Term	Definition
Free radicals	An atom or a group of atoms with an unpaired electron, which are chemically active to initiate a chain reaction via catching another electron from other chemicals, and most free radicals contain an oxygen atom.
Oxidative stress	Disturbance in the pro-oxidant–antioxidant balance caused by overproduction or impaired removal of free radicals or reactive oxygen species, which results in potential damage to cells.
Endothelial dysfunction	Abnormal functions of the endothelial layer in blood vessels, such as blood flow, vessel constriction and dilation, which are associated with oxidative damage to the endothelial cells, and known as a risk factor for cardiovascular disease.
Hyperlipidaemia	Elevation in blood lipids, including cholesterol, cholesterol esters, phospholipids and/or triglycerides, which is an important risk factor for cardiovascular disease.

Status in Smokers

Tocopherol status in smokers

Impaired antioxidant status, such as plasma β-carotene and ascorbate in most studies, was observed in smokers (Duthie *et al.*, 1989; Faruque *et al.*, 1995; Mezzetti *et al.*, 1995; Ross *et al.*, 1995; Marangon *et al.*, 1998; Liu *et al.*, 2000; Kim and Lee, 2001; Dietrich *et al.*, 2003; Valachovicova *et al.*, 2003). However, few studies reported impaired α-tocopherol status in smokers. An *in vitro* study showed loss of α- and γ-tocopherol by 70% ($P < 0.001$) and 30% ($P < 0.05$), respectively, in fresh normal human plasma exposed to gas phase cigarette smoke at 9 h (Handelman *et al.*, 1996). The plasma α-tocopherol concentration in smokers (1.68 ± 0.48 μg/mg total lipid) was significantly lower ($P < 0.05$) than that in non-smokers (2.78 ± 1.09 μg/mg total lipid) (Liu *et al.*, 2000). Zhou *et al.* (2000) also found that plasma α-tocopherol decreased significantly ($P < 0.001$) in smokers compared with non-smokers. A linear regression and correlation analysis for 40-year-old male smokers showed that the longer the duration of smoking and the greater daily quantity of smoking, the lower was the value of plasma α-tocopherol. The plasma α-tocopherol concentration increased markedly in smokers 1–6 months after smoking cessation, suggesting that a reduced plasma α-tocopherol level is associated with smoking (Liu *et al.*, 2000; Zhou *et al.*, 2000; Polidori *et al.*, 2003). The level of α-tocopherol in alveolar fluid of smokers was relatively lower compared with that in alveolar fluid of non-smokers (3.1 ± 0.7 *versus* 20.7 ± 2.4 ng/ml, $P < 0.005$) (Pacht *et al.*, 1986). Additionally, the α-tocopherol level in the internal mammary artery was significantly lower ($P < 0.0006$) in smokers than in non-smokers (Mezzetti *et al.*, 1995). However, some studies found that α-tocopherol levels in the blood and erythrocytes did not differ significantly between smoking and non-smoking males (Duthie *et al.*, 1989; Hoshino *et al.*, 1990; Faruque *et al.*, 1995; Mezzetti *et al.*, 1995; Ross *et al.*, 1995; Brown *et al.*, 1996; Dietrich *et al.*, 2003; Valachovicova *et al.*, 2003). Smokers and passive smokers had a significantly higher plasma γ-tocopherol concentration, but not α-tocopherol, than non-smokers (7.8, 7.8 and 6.5 μmol/l, respectively) after adjustment for dietary antioxidant intake (Dietrich *et al.*, 2003). Moderate (<20 cigarettes/day) and heavy (>20 cigarettes/day) smokers did not have different plasma α-tocopherol levels from those of non-smokers (Marangon *et al.*, 1998). Smokers even had higher α-tocopherol levels in the serum and bronchoalveolar cells than non-smokers (Hilbert and Mohsenin, 1996). Additionally, ex-smokers had significantly higher plasma α-tocopherol level than non-smokers (Marangon *et al.*, 1998) (Table 26.2). These findings suggest that systematic α-tocopherol status, such as circulating α-tocopherol, may be impaired or not affected in smokers, but the local α-tocopherol level may be more sensitive to being altered in an adaptive manner in order to participate in antioxidant defences.

Table 26.2. Tocopherol status in smokers.

Compartment	Study condition	Tocopherol status	References
Blood	9 h exposure to gas-phase cigarette smoke	↓ α- and γ-tocopherol	Handelman *et al.* (1996)
Blood	Smokers versus non-smokers	↓ α- tocopherol	Liu *et al.* (2000); Zhou *et al.* (2000)
Blood	Smokers versus non-smokers	↑ α- tocopherol	Hilbert and Mohsenin (1996)
Blood	Smokers versus non-smokers	↑ γ-tocopherol	Dietrich *et al.* (2003)
Blood	Ex-smokers versus non-smokers	↑ α- tocopherol	Marangon *et al.* (1998)
Blood	Smoking cessation	↑ α- tocopherol	Liu *et al.* (2000); Zhou *et al.* (2000); Polidori *et al.* (2003)
Blood and erythrocytes	Smokers versus non-smokers	No change in α-tocopherol level	Duthie *et al.* (1989); Hoshino *et al.* (1990); Faruque *et al.* (1995); Mezzetti *et al.* (1995); Ross *et al.* (1995); Brown *et al.* (1996); Marangon *et al.* (1998); Dietrich *et al.* (2003); Valachovicova *et al.* (2003)
Alveolar fluid	Smokers versus non-smokers	↓ α- tocopherol	Pacht *et al.* (1986)
Mammary artery	Smokers versus non-smokers	↓ α- tocopherol	Mezzetti *et al.* (1995)
Bronchoalveolar cells	Smokers versus non-smokers	↑ α- tocopherol	Hilbert and Mohsenin (1996)

The effect in smokers may be due to less intake of dietary tocopherol, altered tocopherol kinetics and/or the consumption of tocopherol to scavenge excess free radicals produced by cigarette smoke (Fig. 26.1). Current smokers had a lower intake of dietary tocopherol than non-smokers (4.05 *versus* 4.72 mg/1000 kcal, $P < 0.05$) in the UK (Dyer *et al.*, 2003). Similarly, smokers consumed less dietary tocopherol ($P < 0.05$) compared with non-smokers and ex-smokers in the USA. Female ex-smokers even had a higher intake of dietary tocopherol than female non-smokers (4.82 *versus* 4.54 mg/1000 kcal, $P < 0.05$). Traber *et al.* (2001) demonstrated that smoking increased the plasma disappearance of α-tocopherol. Plasma α-tocopherol tended toward a faster exponential disappearance and shorter half-life in smokers compared with non-smokers after consuming 75 mg each of D- and DL-α-tocopheryl acetate daily for 7 days (0.30 ± 0.04 *versus* 0.24 ± 0.05%, $P = 0.0565$, and 55.6 ± 7.4 *versus* 72.1 ± 17.3 h, $P = 0.0630$, respectively). Furthermore, aggregated free radical production and decreased plasma α-tocopherol level resulted in a disrupted dynamic balance between oxidation and antioxidation accompanied by exacerbated oxidative stress in smokers (Zhou *et al.*, 2000).

Hyperlipidaemia in smokers

Smoking has an adverse effect on low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides in a hypercholesterolaemic population, regardless of gender and age (Schuitemaker *et al.*, 2002). Smokers had higher plasma triglycerides and LDL cholesterol ($P < 0.05$), and lower HDL cholesterol ($P < 0.0005$) and HDL/LDL ratio ($P < 0.05$) compared with non-smokers (Kaçmaz *et al.*, 1997; Kim and Lee, 2001). However, serum triglycerides, total cholesterol, LDL cholesterol and HDL cholesterol did not differ between non-smokers and smokers among patients undergoing aortocoronary by-pass surgery (Mezzetti *et al.*, 1995).

Tocopherol Supplementation in Smokers

Single tocopherol supplementation

Effect on antioxidant status and oxidative damage

Due to increased oxidative stress in smokers, antioxidant supplementation, such as α-tocopherol, has been widely investigated. A short-term study showed that supplementation with 100 or 800 IU/day α-tocopherol in moderate (15–30 cigarettes/day; median, 23 cigarettes/day) or heavy smokers (31–45 cigarettes/day; median, 38 cigarettes/day), respectively, for 5 days failed to suppress urinary excretion of 8-epi-prostaglandin $(PG)F_{2\alpha}$, a stable product of lipid peroxidation *in vivo* (Reilly *et al.*, 1996). Supplementation with DL-α-tocopheryl acetate (300, 600 and 1200 mg/day) for 3 weeks dose-dependently increased its plasma levels, which reached a plateau at 600 mg (42.3 ± 11.2 μmol/l, $P < 0.001$) (Patrignani *et al.*, 2000). However, tocopherol supplementation did not significantly alter urinary excretion of 8-iso-$PGF_{2\alpha}$ formed non-enzymatically through free radical-catalysed attack on esterified arachidonic acid, and the plasma α-tocopherol level did not correlate with urinary 8-iso-$PGF_{2\alpha}$ excretion ($r = -0.065$, $P = 0.452$). Supplementation with pharmacological doses of tocopherol (300–1200 mg/day) had no detectable effect on thromboxane (Tx) biosynthesis reflected by urinary excretion of its major metabolite, 11-dehydro-TxB_2, in moderate smokers (15–30 cigarettes/day during the previous 2 years). Supplementation with DL-α-tocopherol (800 mg/day) for 14 days in healthy smokers significantly

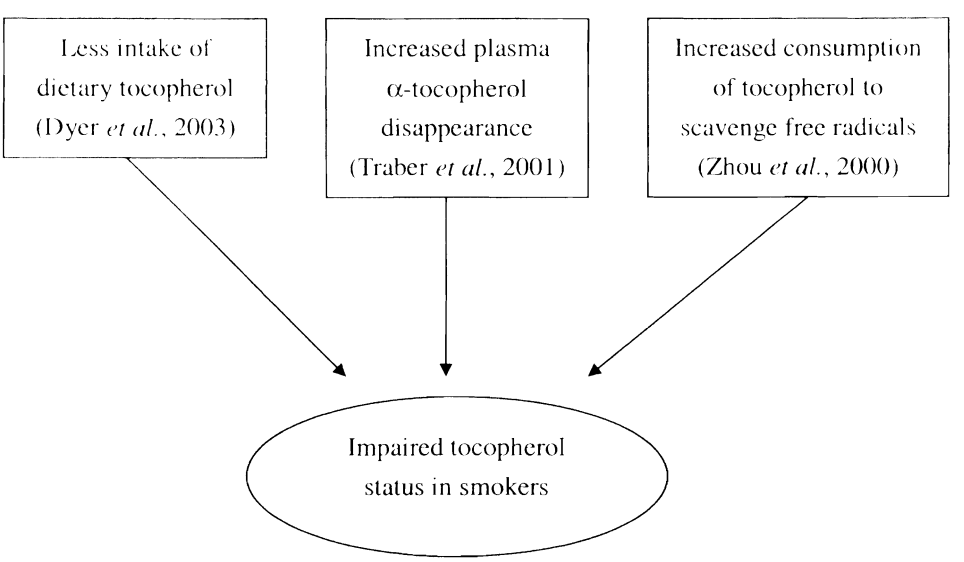

Fig. 26.1. Mechanisms of the impaired tocopherol status in smokers.

decreased breath pentane output, an index of oxidative stress to lipids, and partially restored plasma glutathione peroxidase (GPx) to normal (Hoshino *et al.*, 1990). However, plasma β- and γ-tocopherol levels were reduced significantly in smokers during 14 days supplementation, but were restored to pre-supplementation level 4 weeks after supplement cessation, suggesting that α-tocopherol supplementation can affect the metabolism of other forms of tocopherol. Similarly, Handelman *et al.* (1985) found plasma β- and γ-tocopherol levels to be decreased in healthy non-smokers after supplementation with DL-α-tocopherol (1200 IU/day) for 8 weeks, and suggested that intestinal uptake and/or plasma transport used α-tocopherol more efficiently than β- or γ-tocopherol. Supplementation with 1000 mg/day α-tocopheryl acetate for 14 days significantly elevated plasma and erythrocyte α-tocopherol levels to three- and twofold ($P < 0.001$), respectively, and decreased erythrocyte hydrogen peroxide (H_2O_2)-induced peroxidation ($P < 0.001$) in smokers (Duthie *et al.*, 1989). However, tocopherol supplementation did not affect increased plasma conjugated dienes and erythrocyte reduced glutathione, decreased plasma ascorbate and glucose-6-phosphate dehydrogenase activity caused by smoking. However, tocopherol supplementation (800 IU/day; 800 mg/day DL-α-tocopheryl acetate) for 3 weeks in smokers consuming 20% safflower oil high in linoleic acid significantly increased total F_2-isoprostanes and $PGF_{2\alpha}$ by 62 and 42% ($P < 0.001$), respectively, although it prolonged the lag time of LDL oxidation by 23% ($P = 0.006$) compared with that in smokers without tocopherol supplementation (Weinberg *et al.*, 2001). The data suggest that α-tocopherol may act as a pro-oxidant in smokers consuming a high polyunsaturated fat diet. Tocopherol supplement (600 IU/day) for 4 weeks normalized the increased plasma thiobarbituric acid-reactive substances (TBARS) but not plasma total oxysterols in smokers (Mol *et al.*, 1997). Porkkala-Sarataho *et al.* (1998) found that supplementation with D-α-tocopheryl acetate (200 mg/day) for 2 months in male smokers significantly elevated plasma and very low-density lipoprotein (VLDL) + LDL α-tocopherol concentrations by 88% ($P < 0.0001$) and 90% ($P < 0.0001$), and the LDL total peroxyl radical-trapping antioxidant parameter by 58% ($P < 0.0001$). Additionally, the lag time of oxidation in the VLDL + LDL fraction was prolonged by 34 and 109% ($P < 0.0001$), assessed using copper- and haemin + H_2O_2-induced methods, respectively, and the time to maximal oxidation was prolonged by 21% ($P = 0.001$) measured by the copper-induced method in smokers receiving α-tocopherol supplementation. Similarly, both plasma and LDL α-tocopherol levels were elevated significantly to twofold ($P < 0.01$), and the lag phase of Cu^{2+}-catalysed LDL oxidation increased significantly by 64% (week 0, 118 ± 31 *versus* week 8, 193 ± 80 min, $P < 0.05$), whereas the LDL oxidation rate and the production of superoxide anion in polymorphonuclear leukocytes and conjugated dienes in LDL did not change in smokers supplemented with D-α-tocopheryl acetate (400 IU/day; 294 mg/day) for 8 weeks (Fuller *et al.*, 2000). A higher dose of α-tocopherol (900 IU/day) significantly decreased luminol-enhanced chemiluminescence responses of activated phagocytes by *N*-formylmethionyl-leucylphenylalanine and cytochalasin B ($P < 0.005$) after 4 and 6 weeks supplementation in smokers, indicating that tocopherol supplementation inhibited the generation of oxidants by activated phagocytes (Richards *et al.*, 1990). The erythrocyte tocopherol concentration and catalase activity increased significantly by 92 and 13% ($P < 0.001$), respectively, and H_2O_2-induced lipid peroxidation was abolished significantly by 54% ($P < 0.001$) after supplementation with DL-α-tocopheryl acetate (280 mg/day) for 10 weeks in smokers (Brown *et al.*, 1996). However, increased erythrocyte superoxide dismutase (SOD), GPx and glutathione reductase activities causd by smoking did not change significantly with tocopherol supplementation. Although α-tocopherol supplementation eliminated lipid peroxidation in smokers, daily supplementation with 200 mg of D-α-tocopheryl acetate in smokers for 2 months did not affect the urinary excretion rate of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), a repair product of oxidative DNA damage (Prieme *et al.*, 1997).

Whereas high dose supplementation with tocopherol (2400 IU/day) for 3 weeks increased the tocopherol level threefold in the alveolar fluid of smokers compared with that before supplementation (3.1 ± 0.7 *versus* 9.3 ± 2.3 ng/ml), it remained relatively lower ($P < 0.001$) than the baseline level of non-smokers (Pacht *et al.*, 1986). Additionally, the killing of normal rat lung parenchymal cells by alveolar macrophages of smokers was inversely related to the tocopherol content of the parenchymal cells, suggesting that tocopherol may be an important antioxidant for the lower respiratory tract in the defence against oxidative injury.

A long-term study showed that the erythrocyte tocopherol concentration increased in a dose-dependent manner during 20 weeks of daily supplementation with 70, 140, 560 and 1050 mg of D-α-tocopherol in smokers with habitually low ascorbate and α-tocopherol intake (Brown *et al.*, 1997). Each dose of tocopherol supplements was associated with a significant decrease (61–73%) in susceptibility of erythrocytes to H_2O_2-induced lipid peroxidation in smokers ($P < 0.001$). Supplementation with DL-α-tocopherol (400 IU/day; 364 mg/day) for 2 years also decreased plasma TBARS and *in vitro* LDL oxidation in smokers (van Tits *et al.*, 2001) (Table 26.3).

Effect on endothelial function of blood vessels

Tocopherol supplementation may affect transient impairment of endothelial function after heavy cigarette smoking, and chronic endothelial dysfunction in smokers results at least in part from increased oxidative stress (Neunteufl *et al.*, 2000). Flow-mediated vasodilation (FMD) values remained similar in male smokers supplemented with α-tocopherol (600 IU/day) for 4 weeks compared with those receiving placebo, but decreased more quickly after smoking a cigarette in subjects taking placebo compared with those receiving α-tocopherol ($P = 0.0001$ and 0.001 for time and group factors, respectively). Furthermore, the transient attenuation of changes in FMD was related to the improvement of the antioxidant status, estimated as percentage changes in TBARS ($r = -0.67$, $P = 0.0024$).

Therefore, Neunteufl *et al.* (2000) suggest that α-tocopherol supplementation can attenuate transient impairment of endothelial function after heavy smoking due to enhanced oxidative status, but cannot restore chronic endothelial dysfunction within 4 weeks in healthy male smokers. After 4 weeks supplementation with α-tocopherol (1000 IU/day), forearm blood flow responses to noradrenaline or NG-monomethyl-L-arginine, the competitive inhibitor of nitric oxide synthesis, remained unchanged in healthy smokers, indicating that α-tocopherol supplementation does not affect the basal activity of the noradrenaline-constrictor and nitric oxide-dilator systems *in vivo* (Green *et al.*, 1995). However, a double-blind, placebo-controlled study showed that long-term (4 months) supplementation with D-α-tocopheryl acetate (544 IU/day; 400 mg/day) significantly increased endothelium-dependent relaxation in hypercholesterolaemic smokers but not in hypercholesterolaemic non-smokers or normocholesterolaemic chronic smokers (Heitzer *et al.*, 1999). Additionally, higher autoantibody levels against oxidized LDL in hypercholesterolaemic smokers, as compared with those in hypercholesterolaemic non-smokers or normocholesterolaemic chronic smokers, significantly decreased after 4 months α-tocopherol supplementation. These results indicate that long-term α-tocopherol supplementation improves endothelium-dependent relaxation in forearm vessels of hypercholesterolaemic smokers. Overall, the beneficial effect of α-tocopherol supplementation alone on the impaired endothelial function of vessels may be more noticeable in smokers with increased oxidized LDL or oxidative stress for a longer duration of supplementation, but not in healthy smokers (Table 26.4).

Effect on the incidence of diseases

Recently, the relationship between tocopherol supplementation alone and certain diseases in smokers has been studied. A 5–8 year (median, 6 years) follow-up, randomized, double-blind, placebo-controlled primary prevention trial – the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study (ATBC study) – found that supplementation with DL-α-tocopherol (50 mg/day) in male

Table 26.3. Effects of single tocopherol supplementation on antioxidant status and oxidative damage in smokers.

Tocopherol dosage	Study duration	Results	References
100 or 800 IU/day α-tocopherol	5 days	No change in urinary 8-iso-$PGF_{2\alpha}$ excretion	Reilly *et al.* (1996)
800 mg/day DL-α-tocopherol	14 days	↓ breath pentane output ↓ plasma β- and γ-tocopherol ↑ plasma GPx	Hoshino *et al.* (1990)
1000 mg/day α-tocopheryl acetate	14 days	↑ plasma and erythrocyte α-tocopherol ↓ erythrocyte lipid peroxidation	Duthie *et al.* (1989)
300–1200 mg/day DL-α-tocopheryl acetate	21 days	↑ plasma α-tocopherol No changes in urinary excretion of 8-iso-$PGF_{2\alpha}$ and 11-dehydro-TXB_2	Patrignani *et al.* (2000)
800 IU/day DL-α-tocopheryl acetate + 20% safflower oil	21 days	↑ total F_2-isoprostanes and $PGF_{2\alpha}$ ↑ lag time of LDL oxidation	Weinberg *et al.* (2001)
2400 IU/day α-tocopherol	21 days	↑ alveolar fluid α-tocopherol	Pacht *et al.* (1986)
600 IU/day α-tocopherol	28 days	↓ plasma TBARS	Mol *et al.* (1997)
900 IU/day α-tocopherol	4 or 6 weeks	↓ phagocyte oxidation	Richards *et al.* (1990)
200 mg/day D-α-tocopheryl acetate	8 weeks	↑ plasma and VLDL + LDL α-tocopherol ↑ LDL total peroxyl radical trapping antioxidant parameter	Porkkala-Sarataho *et al.* (1998)
		↑ Lag time of VLDL + LDL oxidation	Prieme *et al.* (1997)
200 mg/day D-α-tocopheryl acetate	8 weeks	No change in DNA oxidation	
400 IU/day D-α-tocopheryl acetate	8 weeks	↑ plasma and LDL α-tocopherol ↑ lag time of LDL oxidation	Fuller *et al.* (2000)
280 mg/day DL-α-tocopheryl acetate	10 weeks	↑ erythrocyte α-tocopherol ↑ erythrocyte catalase ↓ erythrocyte lipid peroxidation	Brown *et al.* (1996)
70–1050 mg/day D-α-tocopherol	20 weeks	↑ erythrocyte α-tocopherol ↓ erythrocyte lipid peroxidation	Brown *et al.* (1997)
400 IU/day DL-α-tocopherol	2 years	↓ plasma TBARS ↓ LDL oxidation	van Tits *et al.* (2001)

Table 26.4. Effects of single tocopherol supplementation on endothelial function of blood vessels in smokers.

Tocopherol dosage	Study duration	Results	References
600 IU/day α-tocopherol	28 days	No change in flow-mediated vasodilation	Neunteufl *et al.* (2000)
1000 IU/day α-tocopherol	28 days	No changes in forearm blood flow responses to constrictor and dilator	Green *et al.* (1995)
544 IU/day D-α-tocopheryl acetate	4 months	↑ endothelium-dependent relaxation in hypercholesterolaemic smokers	Heitzer *et al.* (1999)

smokers from southwestern Finland did not reduce the incidence of lung cancer (−2%, 95% confidence interval (CI) = −14–12%) and total mortality, although there were more deaths from haemorrhagic stroke (Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group, 1994). Tocopherol supplementation alone from the ATBC study increased the risk of subarachnoid haemorrhage 50% (95% CI = −3–132%, $P = 0.07$; relative risk (RR) = 2.45, 95% CI = 1.08–5.55) and decreased the risk of cerebral infarction 14% (95% CI = −25 to −1%, $P = 0.03$; RR = 0.70, 95% CI = 0.55–0.89) in hypertensive male smokers but had no effect in normotensive male smokers (Leppala *et al.*, 2000a, b). Additionally, tocopherol supplementation significantly decreased the risk of cerebral infarction (RR = 0.33, 95% CI = 0.14–0.78), without increasing the risk of subarachnoid haemorrhage, in hypertensive male smokers with diabetes, indicating that tocopherol supplementation may prevent ischaemic stroke in high-risk hypertensive patients (Leppala *et al.*, 2000a). However, the ATBC study showed that tocopherol supplementation had no significant overall effect on the prevalence of oral mucosal lesions, the cells of unkeratinized epithelium (Liede *et al.*, 1998) or on the common cold in male smokers (Hemila *et al.*, 2002). During a 4 year follow-up period, tocopherol supplementation slightly lowered the incidence of colds among male smokers 65 years of age or older (RR = 0.95, 95% CI = 0.90–1.00), which were reduced the most among older city dwellers who smoked fewer than 15 cigarettes per day (RR = 0.72, 95% CI = 0.62–0.83). An epidemiological cohort study revealed that α-tocopherol supplementation (50 mg/day) for 6.1 years (median) significantly decreased the risk of pneumonia (RR = 0.65, 95% CI = 0.49–0.86) in smokers who had started smoking at a later age (≥21 years, $n = 7469$ with 196 pneumonia cases) (Hemila *et al.*, 2004). However, tocopherol supplementation had no overall effect on the incidence of pneumonia (RR = 1.00, 95% CI = 0.88–1.14).

Tocopherol cocktail supplementation

Effect on antioxidant status and oxidative damage

Tocopherol antioxidant cocktail has been reported to be beneficial to smokers in enhancing antioxidant status, promoting oxidative resistance and further reducing oxidative damage. A 5 day short-term study found that heavy smokers (31–45 cigarettes/day; median, 38 cigarettes/day) receiving ascorbate (2000 mg/day) and α-tocopherol (800 IU/day) had lower urinary 8-epi-$PGF_{2\alpha}$ excretion (133.4 ± 29.6 *versus* 171.0 ± 39.8 pmol/mmol creatinine, $P < 0.05$), indicating that short-term combined antioxidant therapy can diminish oxidative stress via suppression of the metabolite production of lipid peroxidation (Reilly *et al.*, 1996). After 7 days of supplementation with ascorbate (1000 mg/day) and D-α-tocopherol (500 IU/day; 335.5 mg/day), DNA damage as monitored by the number of micronuclei in peripheral lymphocytes decreased significantly ($P < 0.05$) in smokers (Schneider *et al.*, 2001). Healthy smokers given ascorbate (1000 mg/day) and α-tocopherol (100 mg/day) for 15 days had higher erythrocyte catalase ($P < 0.0005$), SOD ($P < 0.005$) and GPx ($P < 0.005$) activities compared with those before supplementation (Kaçmaz *et al.*, 1997). After antioxidant vitamin supplementation, the formation of TBARS decreased significantly by 52 and 32% ($P < 0.0005$) in the plasma and erythrocytes, respectively. Young smokers receiving ascorbate (1000 mg/day) and D-α-tocopheryl acetate (400 IU/day; 294 mg/day) for 8 weeks increased plasma and LDL α-tocopherol levels twofold ($P < 0.01$), but had a similar plasma ascorbate level after supplementation (Fuller *et al.*, 2000). Additionally, the LDL oxidation rate decreased significantly by 31% ($P < 0.05$) in smokers after combined antioxidant vitamin supplementation, but were not altered after ascorbate or α-tocopherol supplementation alone. However, the DNA oxidation rate measured by the urinary excretion rate of 8-oxodG did not change after supplementation with slow-release ascorbate (500 mg/day) and D-α-tocopheryl acetate (200 mg/day) for 2 months in smokers (Prieme *et al.*, 1997).

Antioxidant vitamin supplementation with β-carotene (22.5 mg/day), ascorbate (1500 mg/day) and α-tocopherol (1200 IU/day) for 6 weeks significantly increased the β-carotene level by 18% ($P < 0.05$) and catalase activity by 55% ($P < 0.05$), but did not significantly affect the tocopherol level in the bronchoalveolar cells of smokers (Hilbert and Mohsenin, 1996). After 6 weeks supplementation with antioxidant vitamins, plasma β-carotene, ascorbate, α-tocopherol and erythrocyte glutathione levels increased significantly by 200, 98, 129 and 39%, respectively, in the intervention I group (15 mg β-carotene/day + 500 mg ascorbate/day + 400 mg α-tocopherol/day), and by 209, 216, 197 and 32%, respectively, in the intervention II group (30 mg β-carotene/day + 1000 mg ascorbate/day + 800 mg α-tocopherol/day) of hyperlipidaemic smokers (Chao *et al.*, 2002). Except for the erythrocyte GPx activity in the intervention I group, erythrocyte catalase, GPx and SOD activities increased significantly ($P < 0.05$) in both groups. Additionally, the level of ferrous ion in the plasma decreased time dependently ($P < 0.05$), and the ratio of ferrous to ferric ion was reduced significantly by 33 and 39% ($P < 0.05$) in the intervention I and II groups, respectively, which could eliminate oxidative damage from ferrous ion-induced formation of reactive oxygen species. Lipid peroxides in LDL decreased significantly by 56 and 72% ($P < 0.05$) in the intervention I and II groups, respectively. Besides antioxidant cocktail supplementation used in these studies, vitamin-fortified beverages were also made to administer to smokers. After daily consumption of a tomato-based juice supplemented with β-carotene (30 mg), ascorbate (600 mg) and α-tocopherol (400 mg) for 8 weeks by smokers, plasma β-carotene and α-tocopherol levels increased significantly by 210 and 74% ($P < 0.001$), respectively, compared with those in the control group (Steinberg and Chait, 1998). The lag time for the formation of conjugated dienes in LDL of smokers increased significantly by 16% ($P < 0.01$), and the propagation rate decreased significantly by 11% ($P < 0.05$) compared with those in the control group. However, plasma total peroxyl radical trapping potential did not differ significantly between the control and antioxidant vitamin-supplemented

groups. A long-term study showed that supplementation with an antioxidant cocktail containing β-carotene (30 mg/day), ascorbate (400 mg/day), D-α-tocopheryl acetate (200 mg/day) and organic selenium (100 μg/day) for 3 months in smokers significantly increased plasma β-carotene by 209%, ascorbate by 45%, α-tocopherol by 72% and selenium by 20% ($P < 0.001$) (Nyyssönen *et al.*, 1994). The concentrations of β-carotene and α-tocopherol in the VLDL + LDL fraction was also significantly elevated by 223 and 70% ($P < 0.001$), respectively, these values being close to the increases in the plasma. The lag time to oxidation of the VLDL + LDL fraction increased by 27% ($P < 0.001$) and 29% ($P = 0.002$), and the maximal oxidation velocity decreased by 10% ($P < 0.05$) and 15% ($P = 0.07$) after copper and haemin + H_2O_2 induction, respectively, in the antioxidant-supplemented group compared with those in the placebo group (Table 26.5).

Effect on endothelial function of blood vessels

Tocopherol antioxidant cocktail may be beneficial to smokers in improving vasodilation and reducing the inflammatory response via mediating vascular endothelial functions. The forearm vasodilatory response to reactive hyperaemia as an index of endothelium-dependent dilation increased significantly ($P < 0.05$) in healthy young smokers supplemented with ascorbate (2000 mg/day) and tocopherol (400 or 800 IU/day) for 4 weeks (Antoniades *et al.*, 2003; Tousoulis *et al.*, 2003). Administration of ascorbate (2000 mg/day) and tocopherol (800 IU/day) for 4 weeks also significantly decreased serum or plasma levels of interleukin-1β (IL-1β), IL-6, soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble intercellular adhesion molecule-1 (sICAM-1), plasminogen activator inhibitor (PAI-1), von Willebrand factor (vWF) and the ratio of PAI-1 to tissue plasminogen activator in smokers ($P < 0.05$), suggesting combined ascorbate and tocopherol may improve endothelial functions via regulation of inflammatory and thrombosis/fibrinolysis responses. However, a long-term randomized double-blind placebo-controlled study found that supplementation with DL-α-tocopherol (400 IU/day; 364 mg/day) alone for 2 years did not affect the plasma sICAM-1 level, which increased significantly by 42% compared with that of non-smokers, in male normolipidaemic chronic smokers (van Tits *et al.*, 2001) (Table 26.6).

Effect on blood lipids

Although use of a tocopherol antioxidant cocktail in smokers improves antioxidative capacity, blood lipids may not obviously change. The levels of triglycerides, total

Table 26.5. Effects of tocopherol cocktail supplementation on antioxidant status and oxidative damage in smokers.

Tocopherol dosage	Study duration	Results	References
2000 mg/day ascorbate + 800 IU/day α-tocopherol	5 days	↓ urinary 8-epi-$PGF_{2\alpha}$ excretion	Reilly *et al.* (1996)
1000 mg/day ascorbate + 500 IU/day D-α-tocopherol	7 days	↓ DNA damage	Schneider *et al.* (2001)
1000 mg/day ascorbate + 100 mg/day α-tocopherol	15 days	↑ erythrocyte catalase and GPx ↓ plasma and erythrocyte TBARS	Kaçmaz *et al.* (1997)
500 mg/day ascorbate + 200 mg/day D-α-tocopheryl acetate	8 weeks	No change in DNA oxidation rate	Prieme *et al.* (1997)
1000 mg/day ascorbate + 400 IU/day D-α-tocopheryl acetate	8 weeks	↑ plasma and LDL α-tocopherol ↓ LDL oxidation rate	Fuller *et al.* (2000)
15 mg/day β-carotene + 500 mg/day ascorbate + 400 mg/day α-tocopherol	6 weeks	↑ plasma β-carotene, ascorbate and α-tocopherol ↑ erythrocyte glutathione ↑ erythrocyte catalase and SOD ↓ plasma ferrous ion ↓ LDL lipid peroxidation	Chao *et al.* (2002)
30 mg/day β-carotene + 1000 mg/day ascorbate + 800 mg/day α-tocopherol	6 weeks	↑ plasma β-carotene, ascorbate and α-tocopherol ↑ erythrocyte glutathione ↑ erythrocyte catalase, GPx and SOD activities ↓ plasma ferrous ion ↓ LDL lipid peroxidation	Chao *et al.* (2002)
22.5 mg/day β-carotene + 1500 mg/day ascorbate + 1200 IU/day α-tocopherol	6 weeks	↑ bronchoalveolar β-carotene ↑ bronchoalveolar catalase activity No change in bronchoalveolar α-tocopherol	Hilbert and Mohsenin (1996)
30 mg/day β-carotene + 600 mg/day ascorbate + 400 mg/day α-tocopherol in tomato juice	8 weeks	↑ plasma β-carotene and α-tocopherol ↑ lag time of LDL oxidation ↓ propagation rate of LDL oxidation	Steinberg and Chait (1998)
30 mg/day β-carotene + 400 mg/day ascorbate + 200 mg/day D-α-tocopheryl acetate + 100 μg/day organic selenium	3 months	↑ plasma β-carotene, ascorbate, α-tocopherol and selenium ↑ VLDL + LDL β-carotene and α-tocopherol ↑ lag time of VLDL + LDL oxidation ↓ maximal oxidation velocity of VLDL + LDL oxidation	Nyyssönen *et al.* (1994)

Table 26.6. Effects of tocopherol cocktail supplementation on endothelial function of blood vessels in smokers.

Tocopherol dosage	Study duration	Results	References
2000 mg/day ascorbate + 400 or 800 IU/day tocopherol	4 weeks	↑ endothelium-dependent dilation	Antoniades *et al.* (2003); Tousoulis *et al.* (2003)
2000 mg/day ascorbate + 800 IU/day tocopherol	4 weeks	↓ blood IL-1β, IL-6, sVCAM-1, sICAM-1, PAI-1 and vWF ↓ blood PAI-1/tissue plasminogen activator	Antoniades *et al.* (2003); Tousoulis *et al.* (2003)

IL, interleukin; sVCAM-1, soluble vascular adhesion molecule-1; sICAM-1, soluble intercellular adhesion molecule-1; PAI-1, plasminogen activator inhibitor-1; vWF, von Willebrand factor.

cholesterol, LDL cholesterol and HDL cholesterol in the blood of smokers with or without hyperlipidaemia were not affected by single or combined tocopherol supplements (Nyyssönen *et al.*, 1994; Porkkala-Sarataho *et al.*, 1998; Steinberg and Chait, 1998; Wu *et al.*, 1999; Fuller *et al.*, 2000). After supplementation with ascorbate (1000 mg/day) and α-tocopherol (100 mg/day) for 15 days in healthy young smokers, plasma HDL cholesterol and the HDL/LDL ratio increased significantly by 15% ($P < 0.01$) and 59% ($P < 0.05$), and LDL cholesterol decreased significantly by 30% ($P < 0.0005$) compared with those before antioxidant vitamin supplementation (Kaçmaz *et al.*, 1997).

Conclusions

Smokers have impaired antioxidant status, lipid profiles and endothelial functions due to increased oxidative stress, as compared with non-smokers. Tocopherol supplementation improves antioxidant status and endothelial functions, enhances oxidative resistance and further reduces oxidative damage in smokers. Tocopherol antioxidant cocktail has more observable evidence of antioxidant defensive ability against oxidative damage compared with single tocopherol supplementation (Fig. 26.2).

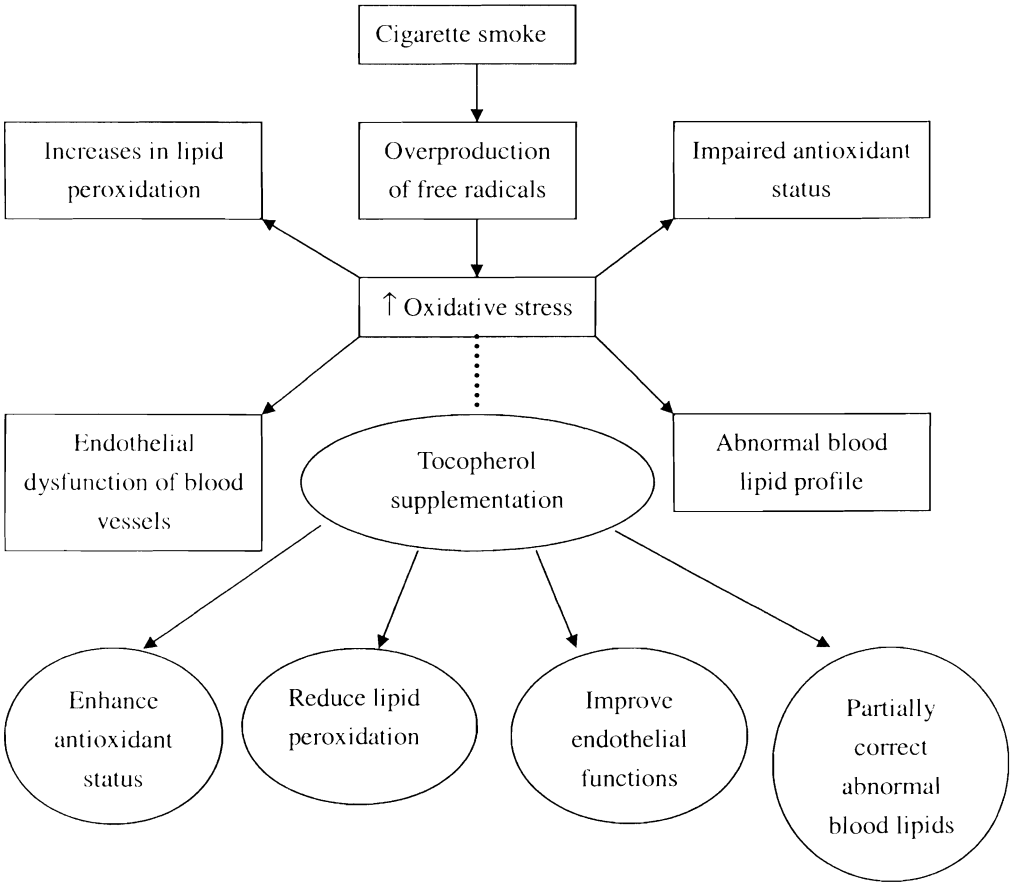

Fig. 26.2. Overall effects of tocopherol supplementation in smokers.