Eur J Nutr 41 : 27–34 (2002)
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ORIGINAL CONTRIBUTION

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Vitamin C and vitamin E antagonistically modulate human vascular endothelial and smooth muscle cell DNA synthesis and proliferation

Summary *Background* Vitamin C and E are suggested to play a preventive role in the pathogenesis of atherosclerosis. They reduce oxidation of low density lipoproteins (oxLDL), thereby protecting human vascular arterial endothelial and smooth muscle cells from oxLDL induced damages. *Aims of the Study* Since vascular arterial endothelial and smooth muscle cells are both involved in atherosclerotic plaque formation, we simultaneously examined the effect of vitamin C, E and oxLDL on their DNA synthesis and proliferation to further elucidate their joint role in this process. *Methods* Human umbilical arterial endothelial cells (HUAEC) and human arterial smooth muscle cells (HUASMC) were incubated with "preventive concentrations" of vitamin C (60 µM) and E (30 µM) and with LDL (60 µg/ml) of increasing oxidation grade. Cell proliferation and DNA synthesis were determined by cell count and [^{3}H]-thymidine uptake, respectively. *Results* Vitamin C alone or in combination with E increased significantly cell number and [^{3}H]-thymidine uptake in HUAEC. The combination exhibited the strongest effect. In contrast, cell number and [^{3}H]-thymidine uptake in HUASMC were significantly decreased in the presence of vitamin C, vitamin E or its combination. OxLDL (60 µg/ml) inhibited cell number and [^{3}H]-thymidine uptake in HUAECs, the latter in an oxidation-grade dependent manner. In HUASMC oxLDL promoted a higher cell number and [^{3}H]-thymidine uptake. If induced by minimally oxLDL, this reduction or increase could be partially reversed by vitamin C alone or in combination with vitamin E. *Conclusion* Vitamin C and E, alone or in combination, modulate proliferation and DNA synthesis of human arterial endothelial and muscle cells and this modulation is antagonistic. Thus, vitamin C and E may act "preventive" on atherosclerotic plaque formation in two steps: first reendothelialisation is promoted, then HUASMC growth is inhibited.

Key words Reendothelialisation – smooth muscle cells – lipoproteins – vitamin E and C

Received: 23 August 2001
Accepted: 8 January 2002

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Introduction

The lipophilic vitamin E and the hydrophilic vitamin C are suggested to play a preventive role in atherosclerosis. Low blood levels of vitamin C and E have been associated with an increased risk of coronary artery disease (CAD) [1–3] and their increased intake has been shown to be protective [4, 5]. Protection, however, was associated with a high food intake of natural dietary antioxidants while supplementation studies with single antioxidants, especially vitamin E, yielded contradicting results [6–8]. For the protective mechanism, the antioxidative properties of vitamin C and E were mainly investigated. *In vitro* vitamin C and E reduce the oxidative stress towards the tissue in general and specifically reduce the susceptibility of low density lipoproteins (LDL) to oxidation [9, 10]. Oxidised LDL (oxLDL) is attributed a key role in the initiation of atherosclerosis [11]. Evidence supporting such a role includes the presence of oxidised lipids in atherosclerotic lesions [12], the recognition of oxLDL by scavenger receptors, a process which

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can give rise to foam cells [13], the degradation of the endothelial surface layer by oxLDL [14], the cytotoxicity of oxLDL for endothelial cells (ECs) [15], its chemotactic action on monocytes [16] and the acceleration of atherogenesis by the *in vivo* delivery of the gene for 15-lipoxygenase, an oxidising enzyme present in atherosclerotic lesions [17, 18].

But linking the reduced oxidisability of LDL due to antioxidants to a reduction in atherosclerosis may underestimate the involvement of factors in addition to, or other than, LDL in the formation of atherosclerotic lesions as well as additional "non-oxidative" actions of antioxidants. For example, vitamin E is an inhibitor of a) smooth muscle cell proliferation, b) monocyte-endothelial adhesion, c) cytokine release and d) platelet adhesion and aggregation [19, 20].

Vitamin C is an essential cofactor for the formation of collagen and plays a role in protein glycation [21]. It can contribute to an enhancement of carnitine synthesis and the improvement of the plasma-triacylglycerol content [22] and inhibits the DNA adducts induced by synthetic estrogen diethylstilbestrol [23]. The above experimental data have been commonly obtained from one cell system, not seldom non-human, with either vitamin C or E. The aim of this study was to investigate simultaneously the effect of vitamin C and E alone and in combination on DNA synthesis and proliferation of two cell types of the vascular wall – human arterial endothelial cells (HUAEC) and human arterial smooth muscles cells (HUASMC), both isolated from the umbilical cord. OxLDL, as a potential inducer of atherosclerotic lesions, was used to damage the normal cell behaviour and to assess how far both antioxidants could correct this damage. The selected serum concentrations for vitamin C (60 μM) and vitamin E (30μM) are considered to be protective for healthy humans and those suffering from CAD [24–26].

Material and methods

Material

Medium 199, Dulbeccoe's modified Eagle's medium (DMEM), phosphate buffered saline (PBS); HAM's F–10, foetal calf serum (FCS) and trypsin/EDTA were procured from Gibco, collagen type I from rat tail, vitamin C (ascorbic acid), acidic fibroblast growth factor (aFGF), platelet derived growth factor (PDGF), ExtrAvidin peroxidase staining kit stock no. Extra 3, anti-rabbit IgG peroxidase conjugate, anti-human rabbit von Willebrand factor and the monoclonal anti-α-muscle actin mouse antibodies were from Sigma, the fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG antibodies were from Dako, moviol from Hoechst, accutase from PAA Laboratories, GmbH and human fibronectin from Tebu, Frankfurt. Vitamin E (RRR-α-tocopheryl acetate) was a gift of Hoffmann-La Roche, Basel. [^{3}H]-Thymidine was purchased from Amersham. Other chemicals were of analytic grade. Vitamin E was dissolved in a mixture of PBS and dimethylsulfoxide (DMSO). DMSO was used in an end concentration of 0.05 %.

Methods

Primary cultures of human umbilical arterial endothelial cells (HUAEC)

Umbilical cords were collected in PBS (pH 7.4). HUAECs were isolated as described earlier [27] and cultured up to passage 5 at 37 °C on collagen-coated 75 cm^2 flasks in medium 199 supplemented with 20 % FCS, penicillin 100 IU/ml, streptomycin 100 μg/ml, 10 μg/ml heparin and 30 μg/ml crude ECGF in a humidified atmosphere of 5 % CO_2 and 95 % air [27]. Cultures were identified as endothelial cells through their cobble-stone morphology and a positive von Willebrand factor stain by a modified method as described [28]. Cells were fixed in 4 % formalin/PBS for 3 minutes, washed 3 times in PBS, permeabilised with 0.1 % Triton X–100 in PBS for 1 h at RT and washed again with PBS. The von Willebrand factor was detected by an ExtrAvidin Peroxidase staining kit with a primary anti-human von Willebrand factor antibody (1:500) and an anti-rabbit IgG peroxidase conjugate according to manufacturer's description [28].

Primary cultures of human umbilical arterial smooth muscle cells (HUASMC)

HUASMC were isolated from the umbilical arteries by enzymatic digestion [28]. Following the removal of ECs as described, the canulated arteries were incubated with 2 to 3 ml dispase II (0.5 U/mg) mixed with collagenase I (1.2 to 1.6 mg/ml) for a further 60 to 120 minutes. The arteries were purged with 30 ml PBS. The cell-containing PBS was collected, centrifuged and the supernatant was discarded. Cells were resuspended in DMEM, 20 % FCS, plated in 30 mm ø dishes and incubated. SMC attached within 24 h and needed seven to 14 days to reach confluency. Cultures were identified as SMCs by their hill and valley morphology, their negative stain with the Willebrand factor as described for ECs and their immunocytochemical localisation of smooth muscle-specific α-smooth muscle actin. Therefore cells were fixed in 4 % formalin/PBS for 3 min at room temperature (RT), pemeabilised with 0.1 % Triton X-100 in PBS for 1 h, unspecific bindings were blocked with 1 % BSA in PBS for 1 h at RT and the primary anti-human α-actin antibody (1:100) was added over night at 4 °C in a humidified chamber. Cells were washed 6 times with PBS.

The secondary FITC-conjugated F(ab')2 fragments of goat anti-mouse IgG (1:200) were added for 1 h at RT. Cells were washed 6 times with PBS and mounted in moviol. Following an initial subcultivation in a 25 cm^2 flask, cells were propagated in 75 cm^2 flasks coated with human fibronectin (2.4 µg/cm^2) every 3 weeks. Passages 3 to 7 were used for experiments.

[^{3}H]-Thymidine incorporation

HUAEC (5×10^4 cells/well) were seeded in 24-well culture plates coated with collagen (10 µg/well) and cultured in culture medium (see section above). After 24 h, culture medium was removed, cells were washed twice with PBS and quiescient medium (medium 199 without phenol red/HAM's F-10 (1:1, v/v)) was added. After 4 h of incubation, cultures were exposed to vitamin C (60 µM) and E (30 µM) alone or in combination and to LDL (60 µg/ml) for 20 h. For the last 5 h of the 24 h incubation period 2 µCi/ml [^{3}H]-thymidine was added to the quiescient medium. Experiments were terminated as described earlier [27].

HUASMC were seeded into 24-well plates coated with fibronectin (5 µg/well). DMEM/20 % FCS was added. At subconfluency the culture medium was removed, cells were washed with PBS twice and quienscient medium (DMEM/HAMS's F–10 1:1, v/v) was added for 24 h. Thereafter the medium was substituted by quiescent medium containing vitamin C (60 µM) and E (30 µM) alone or in combination and to LDL (60 µg/ml). Cells were incubated for 24 h. During the final 5 h of incubation 2 µCi/ml [^{3}H]-thymidine was added to the medium. Experiments were terminated as described earlier [27]. The amount of fibronectin coating was subtracted from the estimated protein content. Results were calculated as cpm/µg protein and expressed as percentage of the untreated control.

Cell counts

Cell counts were taken with the Casy 1 system (Schärfe System). HUAEC and HUASMC were seeded into 24-well plates and synchronised by the addition of quiescient medium as described in the preceding section. HUAECs and HUASMCs were exposed to vitamin C, E and LDL for 20 or 24 h respectively as described in the preceding section. Thereafter cells were washed twice with PBS (250 µl/well) and detached by a mixture (1:1) of trypsin/EDTA and accutase. Digestion was terminated by the addition of the respected FCS-containing medium (250 µl/well). After centrifugation, cells were resuspended in their respected media and counted with the Casy. The Casy differentiates between viable and dead cells on the basis of the cell size. The given data therefore represent only viable cells.

Since ECs are very fastidious cells, we estimated the lactate dehydrogenase release (LDH) into the supernatant as an additional indicator of cell viability. The LDH release of ECs exposed to vitamin C, E or its combination under the above described experimental conditions were not significantly different from controls (LDH (U/ml): control: 19.9 ± 2.5; vitamin C: 15.7 ± 1.7; vitamin E: 16.4 ± 1.6, vitamin C+E: 15.6 ± 1.8). Thus under the chosen experimental conditions the vitamins are not at all cell toxic.

LDL isolation and modification

LDL (d = 1.019–1.063 g/ml) was isolated from fresh plasma of a normocholesterinemic subject (serum cholesterol ≤ 200 mg/dl, apolipoprotein A 8–10 mg/dl) by KBr-density-gradient ultracentrifugation as described earlier [27] after a period of at least 10 h of fasting. LDL oxidation was promoted by two procedures: LDL was incubated in the presence of $CuSO_4$ (2.5 µmol/l for the oxidation curves (Fig. 1) and 5 µmol/l for cell culture experiments) or exposed to air conditions. Lipid oxidation was monitored photometrically at $\lambda_{234\,nm}$ (conjugated diene formation) as described earlier [27] and at $\lambda_{265\,nm}$ (peroxide formation). Oxidation was terminated by the addition of EDTA (end concentration 1 mM) at different time points. $CuSO_4$ was removed from the LDL preparation by a 6 h dialysis in PBS/1 mM EDTA, pH 7.4 with two

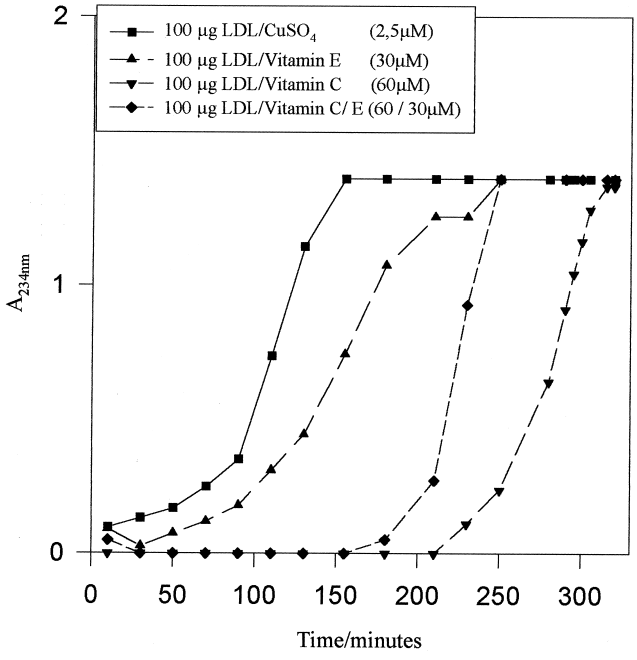

Fig. 1 A typical time course of the copper-promoted oxidation (conjugated diene formation) of the low density lipoprotein (LDL) in the presence or absence of vitamin C and E is shown. Vitamins C and E significantly delayed the copper-promoted oxidation of LDL as evidenced by a longer lag-time. Vitamin C showed the strongest effect. The combination of vitamin C and E took a middle position between the time courses with vitamin E and vitamin C.

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solution exchanges. After sterile filtration LDL was stored at 4 °C in the dark in the presence of 1 mM EDTA up to 3 weeks. Diene and peroxide values were determined before the experiment. LDL concentrations were expressed as protein content. Diene values and peroxide values (in parentheses) of minimally oxidised, oxidised, and highly oxidised LDL (100 µg/ml) ranged between 0.56–0.7 (0.058–0.097); 0.8–0.9 (0.11–0.14) and 1.2–2.4, (0.279–0.615) respectively.

Statistical analysis

Data are presented as mean ± SEM. If not indicated otherwise, a minimum of three independent experiments were performed, each in triplicates with cells of different vessel donors. For comparison the Students' T test was applied in case of normally distributed data, otherwise the Mann-Whitney U test was applied. Differences were considered statistically significant at $p \leq 0.05$. Data analyses were executed on SigmaStat Version 1.0.

Results

In vitro oxidation of LDL

Before vitamin C, E and LDL were applied to the cells, the influence of vitamin C (60 µM) and E (30 µM) alone or in combination on the lag-phase during LDL oxidation was tested *in vitro*. Both antioxidants increased the resistance of LDL to $CuSO_4$-promoted oxidation. Formation of conjugated dienes in the LDL molecule were slowed down. The strongest effect was observed with the hydrophilic vitamin C, the smallest with vitamin E. The combination of vitamin C and E showed an intermediate position between vitamin C and E (Fig. 1).

Effect of vitamin C and E on [^{3}H]-thymidine incorporation and number of viable cells

Vitamin C (60 µM) and E (30 µM) increased the [^{3}H]-thymidine uptake in HUAEC, the first significantly. The combination of both vitamins showed the strongest ef-

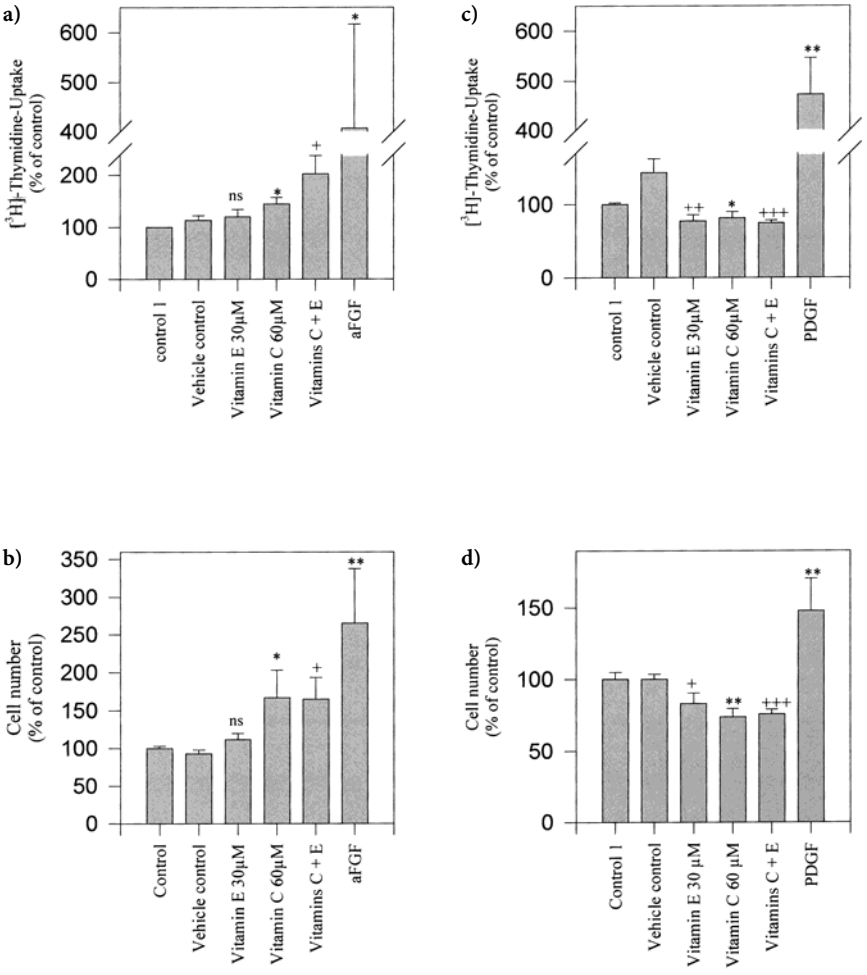

Fig. 2 A 20 h stimulation of HUAEC by vitamin C (60 µM) alone or in combination with vitamin E (30 µM), but not by vitamin E alone, increased the [^{3}H]-thymidine uptake (**a**) and cell number significantly (**b**). The combination increased the [^{3}H]-thymidine uptake clearly more than vitamin C alone. aFGF was used as a positive control for the proliferative capacity. HUAEC of 4 arteries were used. [^{3}H]-Thymidine uptake (**c**) and proliferation of HUASMC (**d**) were inhibited after a 24 h stimulation with either vitamin E, C or their combination. Their [^{3}H]-thymidine uptake and cell number increased in the presence of PDGF as a positive control. HUASMC of 3 arteries were used. In both cell types the observed effects were stronger in the [^{3}H]-thymidine uptake compared to the cell number. Results are presented as percentage of the control/vehicle control. The control relates to vitamin C and the vehicle control, which contained 0.05 % dimethylsulfoxide; relates to solutions containing vitamin E. *:$p \leq 0.05$, ** $p \leq 0.01$ vs control; +: $p \leq 0.05$, ++: $p \leq 0.01$; +++: $p \leq 0.001$ vs vehicle control. *HUAECs* human umbilical arterial endothelial cells, *aFGF* acidic fibroblast growth factor, *HUASMC* human umbilical arterial vascular smooth muscle cells, *PDGF* platelet derived growth factor.

fect and doubled the [^{3}H]-thymidine uptake (Fig. 2a). The increase in the [^{3}H]-thymidine uptake was paralleled by an increase in cell number although the combination of vitamin C and E induced an increase of proliferation comparable to vitamin C (Fig. 2b). In the presence of aFGF, [^{3}H]-thymidine uptake and cell number increased demonstrating thereby the proliferative capacity of the cells.

In contrast, vitamin C and E as well as their combination inhibited the [^{3}H]-thymidine uptake and cell count in HUASMC. The extent of inhibition was most significant for the combination; however for all three conditions the inhibition was only in the range of 20 to 25 % (Fig. 2c,d). PDGF increased [^{3}H]-thymidine uptake and cell count demonstrating the proliferative capacity of the HUASMC under the given experimental conditions.

Effect of oxLDL on [^{3}H]-thymidine incorporation and number of viable cells

With higher grades of oxidation, oxLDL increasingly inhibited the [^{3}H]-thymidine uptake in HUAEC (Fig 3a). On the contrary, HUASMC proliferated in the presence of oxLDL, although there was no clear "dose-response" regarding the oxidation grade of LDL as seen in the HUAEC (Fig. 3b). MoxLDL, oxLDL and highly oxidised LDL (hoxLDL) with conjugated diene values increasing steadily up to 1.2 increased the [^{3}H]-thymidine incorporation to 242 % of the control, whereas the [^{3}H]-thymidine incorporation again dropped to about 145 % in the presence of highly oxLDL with conjugated diene values of 2.4 (hoxLDL(2)). Thus there seems to be a limit to which an increasing oxidation grade of LDL can stimulate the DNA synthesis of HUASMCs. OxLDL significantly decreased the cell number in HUAECs and increased it in HUASMC (Fig. 3c).

Vitamin C and E in combination with minimally oxLDL

The inhibition of the [^{3}H]-thymidine uptake due to minimally oxLDL in HUAEC could be reversed by vitamin C and the combination of C and E (Fig. 4a). The combination was clearly more effective than vitamin C alone. Similarly the minimally oxLDL induced increase of [^{3}H]-thymidine uptake in HUASMC could be reversed by vitamin C or the combination of vitamin C and E (Fig. 4b).

Discussion

LDL oxidation in vitro

Vitamin C and E alone have been shown to protect against Cu^{2+}-induced oxidation of LDL in several *in vitro* and *in vivo* supplementation studies [8–10] and evidence supports that oxidation of LDL by Cu^{2+} plays a

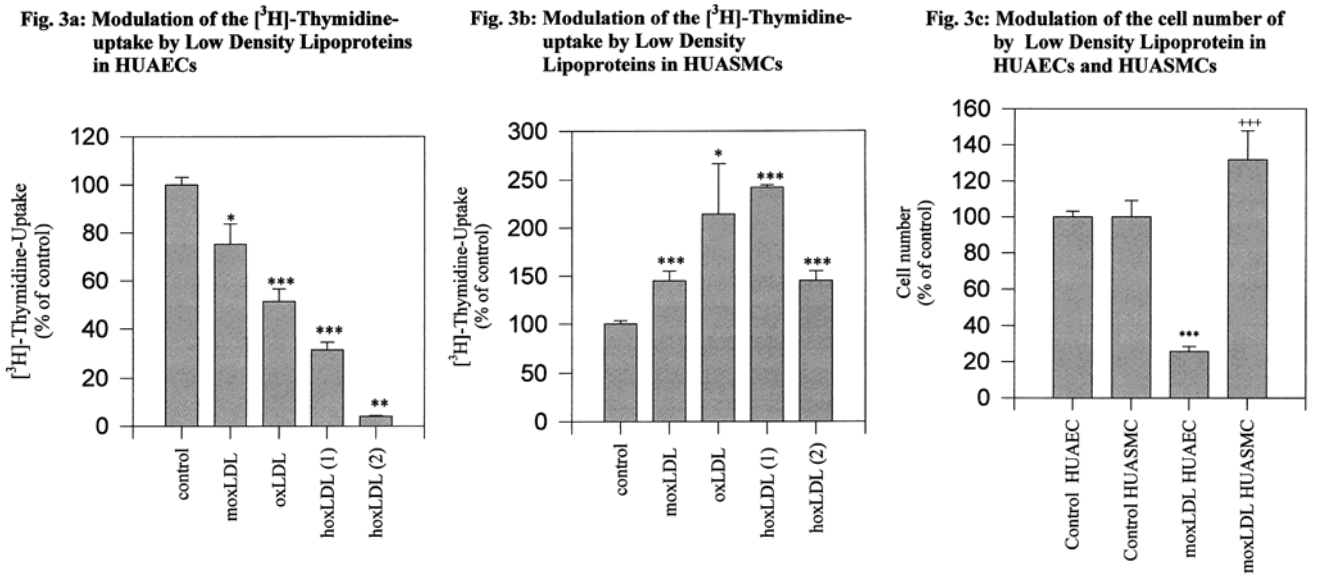

Fig. 3 The [^{3}H]-thymidine uptake decreased in the presence of oxLDL (60 µg/ml) in an oxidation-dependent manner in HUAEC (**a**), and increased in HUASMC independent of the oxidation grade of LDL (**b**). The presence of extremely high oxidised LDL with diene values of 2,4 (hoxLDL2) shows that there is a limit of stimulating [^{3}H]-thymidine uptake in HUASMC further. The same hoxLDL appears already to be toxic for HUAEC. (**c**) Proliferation was inhibited in HUAEC and stimulated in HUASMC by oxLDL. Cells of 3 arteries were used. Data are expressed as percentage of controls. *: p≤0.05, **: p≤0.01, ***: p≤0,001 vs control. +++: p≤0.001 vs moxLDL. hoxLDL(1): $\lambda_{234\ nm}$:1,2; hoxLDL(2): $\lambda_{234\ nm}$: 2.4. (for diene values see Material and methods). *hoxLDL* highly oxidised low density lipoprotein, *moxLDL* minimally oxLDL HUAEC: human umbilical arterial endothelial cells, *HUASMC* human arterial smooth muscle cells.

European Journal of Nutrition, Vol. 41, Number 1 (2002)
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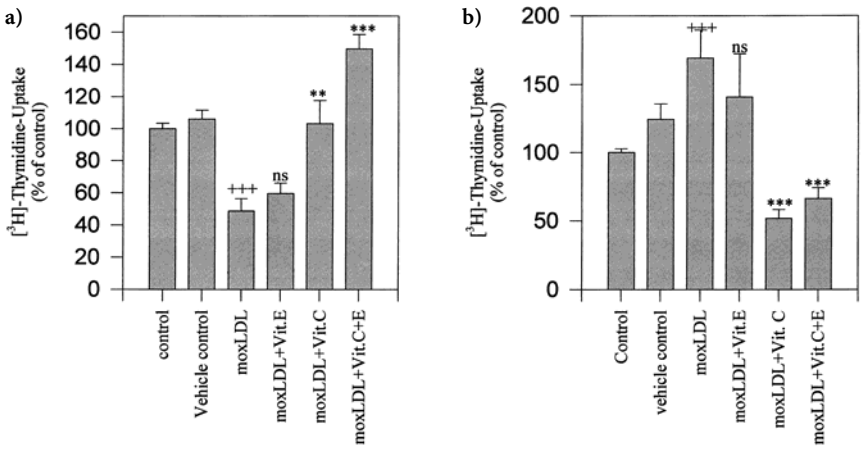

Fig. 4 The effect of minimally oxidised low density lipoprotein (moxLDL) on the [^{3}H]-thymidine uptake could be reversed in HUAEC (**a**) and HUASMC (**b**) by the addition of vitamin C (60 µM) or the combination of vitamin C and E (60 µM/30 µM), but not by vitamin E alone. Cells of 3 arteries were used. Data are expressed as percentage of controls. **: p≤0.01, ***: p≤0.001 vs vehicle control. +++: p≤0.001 vs control. *vit.* vitamin, *HUAEC* human arterial endothelial cells, *HUASMC* human arterial vascular smooth muscle cells.

role *in vivo* [8, 37]. Here, the combination of vitamin C and E (2:1) shows not just an additive effect on LDL oxidation. The middle position of the oxidation curve may reflect differences in the hydrophilic and hydrophobic character of both molecules, but may also relate to the fact that some of the antioxidative vitamin C is needed for the regeneration of vitamin E. Vitamin E radicals have even been proposed to propagate peroxidation within lipoproteins unless they become regenerated back to the unoxidised form by vitamin C. The importance of these pro-oxidative reactions *in vivo* appears questionable [19], but the findings warrant that both – vitamin C and E – should be examined together to assess their physiological actions. Here, the magnitude of the antioxidative action towards LDL depends on the ratio of vitamin C and E in the medium. Nevertheless, it should also be mentioned that antioxidants that inhibit Cu^{2+}-dependent LDL oxidation *ex vivo* may not be effective in lesions in which the predominant LDL oxidation is achieved by other mechanisms [8].

Action of vitamin C and E on HUAEC and HUASMC

The observed experimental results were obtained with the concentrations of vitamin C and E demanded as protective plasma concentrations for healthy humans and those suffering from CAD [24–26]. Although there is evidence for preventive actions of natural dietary antioxidants towards CAD [1–8], it is still unclear, whether the obvious suppression of atherogenesis *in vivo* is related to a suppression of oxidative stress or whether non-oxidative properties of antioxidants may be responsible or add to the effect. We have already previously shown that the combination of vitamin E and C enhances the DNA synthesis of HUAEC [27]. Here we can extend these findings to vitamin C alone and corroborate it with the demonstration that the increase in DNA synthesis is indeed paralleled by an increase in cell number and thus "preventive concentrations" [24] of vitamin C alone or in combination with vitamin E stimulate non-confluent HUAEC cultures to proliferate. We speculate that these mitogenic properties of both vitamins will enhance *in vivo* the reendothelialisation after microdamage. Such a speculation corresponds to findings of Nunes et al. [29] who demonstrated that vitamin C and E promoted vessel remodelling in the coronary artery of pigs. Since a 20 to 25 % increase of lumen area occurred without significant change in the maximal intima thickness, the authors suggested that the primary effect of both antioxidants was an enhancement of outward vessel remodelling. Interestingly, only the combination of vitamin C and E was effective in vessel remodelling. We also observed, contrary to our previous findings [27], no significant effect with vitamin E alone and the highest [^{3}H]-thymidine uptake with the combination in HUAEC. Based on antioxidative mechanisms a possible explanation could be that the lipophilic and therefore mainly membrane-integrated vitamin E needs the water-soluble C for its regeneration. That vitamin E alone can enhance proliferation of ECs of the bovine thoracic aorta, the porcine pulmonary artery [31, 32] or in HUAEC as previously shown, would not contradict such a possibility since species and/or tissue specificities may cause differences in the response to vitamin E. For example, pigs are capable of their own vitamin C synthesis whereas humans are not [29] and cultured HUVEC have low intracellular levels of vitamin C [33].

Injury to the vessel wall does, however, not only affect EC but is also supposed to cause SMC of the media to migrate into the intima, where they proliferate and synthesise an extracellular matrix to form intimal lesions that may impede flow [35]. Vitamin E can inhibit proliferation of human and rat aortic SMC [19, 20]. Here, we demonstrate that this also applies to HUASMC. Vitamin E is proposed to modulate rat and human aortic SMC-proliferation by a non-antioxidant mechanism. Protein kinase C (PKC) is inhibited posttranslational

by dephosphorylation through phosphatase PPA2 [19, 20]. The dephosphorylated PKC-α seems to induce the formation of transcription factor AP1 as well as the transient activation of the α-tropomyosin transcription [19, 20]. How far these mechanisms are active in HUASMC requires further investigation; however it seems likely since reports for rat and human aortic SMC are similar.

That vitamin C inhibits proliferation of human aortic SMC has been shown [36] and our results in HUASMC conform with this. In human aortic SMC this inhibition was paralleled by a reduction of the L-cystein transport and the cellular GSH-levels [36]. More details about the process of inhibition, e. g. which signal transduction pathways are activated, need to be elucidated. We can show here for the first time that the combination of vitamin C and E in a ratio 2:1 and in concentrations as demanded for prevention [24], inhibits HUASMC proliferation with a higher significance but not with a higher magnitude than the single substances. This strengthens the prevailing opinion that the influence on the proliferation of SMC is a non-antioxidant property of vitamin C and E.

OxLDL-induced damages to HUAEC and HUASMC

Although we can demonstrate in HUAECs a direct link between the level of LDL oxidation and the inhibition of DNA synthesis, diene and peroxide values may only be relative figures when appraising oxLDL-induced damages. As seen from Fig. 3a and 4a moxLDL inhibits DNA synthesis in cells originating from different vessel donors to variable magnitudes. In addition to the oxidation of fatty acids, for which the diene and peroxide values are a measure, the protein part of LDL is also subject to oxidation. Our results are related to one LDL-preparation with an identical protein section to which cells of different vessel donors may react distinctly. Responses to oxLDL seem to be influenced by genetic factors. Recently it was demonstrated that genetically varying mouse strains respond distinctly to minimally oxLDL, as judged by the induction of inflammatory monocyte chemotactic protein-1, macrophage stimulating factor, and heme oxygenase-1 [38].

Vitamins C and E were shown to protect human aortic ECs exposed to oxLDL [40, 41]. Vitamin C afforded protection by attenuating adaptive increases in cystein transport and GSH-levels, whereas vitamin E selectively reduced oxLDL induced increases in GSH [40, 41]. Furthermore, in such cells loaded with vitamin E, the oxLDL-induced stimulation of protein kinase C was inhibited [19, 20]. Here we demonstrate protection of HUAECs by vitamin C and the combination C and E through abolishing the inhibition of DNA synthesis. This effect was only seen if minimally oxLDL (moxLDL) was used. However, only moxLDL may be physiologically relevant.

In HUASMC moxLDL does not inhibit, but enhances DNA synthesis. We show here that vitamin C alone or in combination with vitamin E can reverse this accelerated DNA synthesis. Vitamin E has been shown to inhibit an accelerated proliferation of SMC, potentially by an activation of the cellular release of transforming growth factor-ß [20]. However, more details about these protective mechanisms need to be investigated.

In summary, we show here for the first time in a dual experimental set-up that proliferation and DNA synthesis of HUAEC and HUASMC can be modulated antagonistically by vitamin C and E, alone or in combination in the absence or presence of oxLDL. We speculate that this antagonism may play an important role in preserving vascular wall integrity through an agonism in the repair mechanism. The clinical implications may be a general protection of the vessel wall from microdamage in subjects at increased risk of cardiovascular diseases and in the primary prevention of atherosclerosis in general.

Acknowledgement The authors would like to thank the department of gynaecology for their unhesitant cooperation in supplying umbilical cords and A. Ulrich for his frequent readiness to donate blood. Thanks are due to Prof. Dr. B. Gaßmann, Dr. W. Lüder (Potsdam-Rehbrücke, Germany) and Dr. RR. Bhonde (Pune, India) for their critical comments on the manuscript. Authors appreciate the funding of this project by the Doerrenkamp Stiftung (Chur, Switzerland).

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