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Effect of vascular endothelial growth factor and epidermal growth factor on iatrogenic apoptosis in human endothelial cells

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Abstract

To study the effect of growth factors on iatrogenic apoptosis, we examined the influence of vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF) on staurosporine-induced apoptosis in primary cultures of human umbilical vein endothelial cells (HUVEC). Apoptosis was evaluated by a cell viability test, the TUNEL-POD assay and the activation of the pro-apoptotic caspase-3. Staurosporine (10–100 nM) caused the activation of caspase-3. This effect was manifest after 2 hr of incubation and reached its maximum after 5 hr. Severe loss of viability followed within 18 hr. VEGF or EGF (10–100 ng/mL) added together with staurosporine decreased the activation of caspase-3. The loss of viability was 24 hr delayed. The action of growth factors was observed at 1% serum concentration but also at concentration optimal for HUVEC survival (10%, v/v). Furthermore, the inhibition of PI-3 kinase (PI-3K) by wortmannin or LY294002 as well as the inhibition of MEK by PD098059 or U0126 prevented the protective effect of VEGF and EGF. Western blotting analysis showed that after 3 hr of incubation with staurosporine the level of the anti-apoptotic protein Mcl-1 decreased and this effect was reverted by VEGF. It is concluded that VEGF and EGF antagonize the pro-apoptotic action of staurosporine by the combined signalling of PI-3K and ERKs pathways.

Keywords: Apoptosis; Staurosporine; VEGF; EGF; PI-3 kinase; ERKs

1. Introduction

Vascular endothelial cells form the inner layer of blood vessels. Their functional integrity is crucial for the maintenance of blood flow and the antithrombotic activity. To adapt the vasculature to variations of blood flow requirements following physiological and pathological events, the endothelial cells secrete humoral factors that control the relaxation and the contraction of vascular smooth muscle cells, the genesis and dissolution of blood clot, the activation and the inhibition of platelets (reviewed in [1]). When the endothelial function is defective, severe vascular diseases such as atherosclerosis, hypertension and heart failure, may ensue.

According to recent progress [2], a contribution to vascular dysfunctions is given by the programmed cell death, a process known as apoptosis. Apoptosis has been demonstrated in the endothelial cells in normal aging, in the atherosclerotic disease, in the endotoxic shock and in heart failure. Oxidized LDL, circulating tumor necrosis factor, angiotensin II, have been reported to trigger the apoptosis in these cells. A threatening aspect of apoptosis in the endothelial cells is the exposure of phosphatidylserine in the cell surface facing the vascular lumen [3]. The phospholipid polar head may then become the triggering point for the recruitment of factors mediating blood clotting. The detrimental role of apoptosis in the vascular diseases stimulates the search of anti-apoptotic agents. Among the growth factors with anti-apoptotic activity, attention is focused on the VEGF, a peptide involved in the angiogenesis and

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Abbreviations: BSA, bovine serum albumin; EGF, epidermal growth factor; ERKs, extracellular signal-regulated kinases; bFGF, basic fibroblast growth factor; FBS, fetal bovine serum; HUVEC, human umbilical vein endothelial cells; MTT, 3-(4,5-dimethylthiazol-2yl)-2,5,-diphenyltetrazolium bromide; MEK, ERK kinase; PBS, phosphate-buffered saline; PI-3K, phosphatidylinositol-3 kinase; PKC, protein kinase C; VEGF, vascular endothelial growth factor.

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in vascular remodeling. VEGF acts on vascular endothelial cells through two cell surface receptors, VEGFR-1 (Flt-1) and VEGFR-2 (KDR in humans and Flk-1 in mouse) that are connected to several intracellular effectors among which are the focal adhesion kinase, the phosphatidylinositol-3 kinase (PI-3 kinase) and the ERKs [4,5]. The anti-apoptotic effect of VEGF on endothelial cells has been demonstrated during the effect of ionizing radiations [6], the action of tumor necrosis factor-α [7], serum-deprivation [8] and the influence of oxidized LDL [9]. The activation of the PI-3 kinase/Akt signalling pathway and the increased expression of the anti-apoptotic proteins Bcl-2 and A1 are important elements in the VEGF protective action [8,10].

Apoptosis in vascular cells is also a target of pharmacological treatments. Following the observation of an apoptotic effect of the antineoplastic drugs etoposide and doxorubicin on hematopoietic cells [11], it has been reported that doxorubicin [12] and the anticancer drug methylseleninic acid [13] cause apoptosis in the HUVEC. On the basis of these results, we initiated the present study aimed at investigating whether growth factors may protect the human endothelial cells against pharmacological apoptotic stimuli. Support for this hypothesis is found in the observation that VEGF protects the hematopoietic cells from the apoptosis induced by the antineoplastic drugs [11]. In our study, we tested the protective effect of VEGF on the apoptosis induced by staurosporine, a well known inhibitor of cellular protein kinases. Staurosporine and its derivatives cause apoptosis in a variety of mammalian cells and are also under consideration as antineoplastic agents [14]. The effect of VEGF has been compared with that of the EGF, a growth factor that has been shown to suppress staurosporine-induced apoptosis in a human esophageal carcinoma cell line [15]. The data show that both VEGF and EGF protected HUVEC against the pro-apoptotic stimulus induced by staurosporine by signals transmitted through PI-3 kinase and ERKs.

2. Materials and methods

2.1. Chemicals

Cell culture media and supplies were from Sigma, fetal bovine serum (FBS) was from Hyclone. Type 2 collagenase was purchased from Worthington. Human recombinant VEGF, EGF and bFGF were from Sigma. Staurosporine, wortmannin, PD098059 and LY294002 were purchased from Sigma. U0126 was from Calbiochem. TUNEL-POD assay kit and LANCE caspase-3 assay kit were purchased from Roche and Wallac Labelling Service, respectively. Mouse monoclonal antibody to Mcl-1 was provided from Santa Cruz Biotechnology, Inc. Mouse monoclonal antibody against α-tubulin was purchased from Sigma. Horseradish peroxidase-conjugated rabbit anti-mouse IgG was from Amersham. Stock solutions of wortmannin, LY294002, PD098059 and U0126 in dimethylsulfoxide (DMSO) and staurosporine in methanol were kept at −20°.

2.2. Cell culture

HUVEC were isolated in our laboratory as described by Jaffe *et al.* [16] from human umbilical cord obtained during elective cesarean sections. The cells collected were grown on 1% gelatine-coated culture flasks in complete medium M199 containing 10% FBS, 5 ng/mL bFGF, 25 U/mL heparin, 4 mM L-glutamine, 100 U/mL penicillin-G, 100 U/mL streptomycin. HUVEC were used from passages two to six. All the experiments were conducted with different preparations of endothelial cells. HUVEC were grown in complete medium until cultures were confluent. Twenty-four hours before the experiment, cells were trypsinized and plated in 24- or 96-well plate to the subconfluent density of 35,000 cells/cm^2 in complete M199 without bFGF and allowed to settle overnight. Cells were then washed twice with PBS and incubated with or without growth factors in medium M199 supplemented with 10% FBS or 1% FBS plus 1 mg/mL of BSA as indicated.

2.3. MTT assay

The cytotoxic effect of staurosporine was evaluated using the MTT assay. Briefly, the HUVEC were plated in 96-well gelatine-coated tissue culture plate. After the indicated time of treatment, 10 μL of stock solution of MTT (5 mg/mL in PBS) was added to each well. After 4 hr of incubation, the medium was removed and the formazan crystals were dissolved in 200 μL of solution of 0.04 N HCl in isopropanol. The formazan production was time-dependent and proportional to the number of viable cells. MTT reduction was quantified by measuring the light absorbance with a multilabel plate counter (VICTOR^2—Wallac) at 570–630 nm. Background absorbance from control wells (media without cells) was subtracted. The assays were performed in sixtuplicate for each experimental condition.

2.4. Caspase-3 activity

The treated cells were washed twice with PBS and harvested in 80 μL of lysis buffer (10 mM HEPES, 2 mM EDTA, 0.1% CHAPS, 5 mM DTT, pH 7.4). The cells lysate was centrifuged at 12,000 *g* for 10 min and the clear supernatant was used in the analysis. Caspase-3 activity was measured using the LANCE caspase-3 assay kit according to the manufacturer's instruction. The substrate was a tetrapeptide (DEVD) with fluorescent europium chelate coupled to one end and a quencher of europium fluorescence coupled to the other end. The cell extracts (5 μL) were incubated in 10 μL caspase-DTT-reaction-buffer with 200 nM of caspase-3 substrate for 1 hr at 37°. Fluorescence was measured at 340 nm for excitation and 615 nm for emission. Caspase-3

activity was expressed as fluorescence signal normalized for the protein content (Bensadoun and Weinstein procedure [17]).

2.5. TUNEL-POD analysis

HUVEC at 10,000 cells/well in 96-well culture plates were treated for 3 hr with 50 nM of staurosporine with or without VEGF in serum-deficient medium (1% FBS). The apoptotic DNA fragmentation was analyzed using TdT-catalyzed incorporation of fluorescein-labeled nucleotides to free 3′-OH ends of DNA in a template-independent manner using a TUNEL-POD assay kit, according to the manufacture's instructions. *In situ* DNA fragmentation was visualized with a microscope equipped with a digital photocamera.

2.6. Western blotting analysis of Mcl-1 expression

HUVEC were plated in 100-mm dishes at the density of 2×10^6 cells/dish in complete medium. After treatment, cells were lysed in Laemmli buffer, heated for 5 min at 100°, sonicated 30 s and centrifuged at 12,000 *g* for 20 min. The supernatant fluid (40 μg) was subjected to 12.5% SDS–PAGE. Separated proteins were transferred to PDVF membrane (Hybond-P, Amersham) and each membrane was soaked in 5% non fat-dried milk in TBS/Tween-20 (0.1%) at 4° overnight. Membrane were exposed to the primary antibody specific for human Mcl-1 protein (1:100 dilution) in TBS/Tween-20 for 5 hr at room temperature. Blots were then washed three times in TBS/Tween-20 and incubated with horseradish peroxidase-conjugated rabbit anti-mouse IgG (1:15,000 dilution) for 45 min at room temperature. Detection was performed by chemiluminescence using ECL-Plus System (Amersham.). After stripping of each membrane with stripping buffer (100 mM 2-mercaptoethanol, 2% SDS, 62.5 mM Tris–HCl pH 6.7) α-tubulin antibody was used as loading control. Bands were scanned and analyzed with Gelpro-32 densitometer software (version 3.1, Media Cybernetics).

2.7. Statistical analysis

Results are presented as mean ± standard error of the mean (SE). Curves were analyzed by linear regression. Statistical significance of the differences between experimental groups were calculated by unpaired Student's *t*-test. Graphs, EC_{50} determination and statistical analysis were performed using GraphPad Prism version 3.03 for Windows, GraphPad Software.

3. Results

It has been reported that in HUVEC VEGF prevents the apoptosis induced by serum deprivation [8]. To confirm this effect in our cell preparation, we reduced the concentration of serum in the incubation medium from 10 to 1% (v/v). After 18 hr of incubation, the cell viability was reduced by 36% (Fig. 1A) whereas 3 hr of incubation were sufficient to increase the caspase-3 activity by 113% (Fig. 1B), indicating the rapid activation of the caspase-dependent apoptotic pathway. As expected, 50 ng/mL VEGF added during the period of serum deprivation induced a significant protective effect. The action of VEGF was fully manifest between 10 and 100 ng/mL (not shown). Confirming its anti-apoptotic activity, EGF (80 ng/mL) was as active as VEGF in preventing the apoptosis induced by serum deprivation.

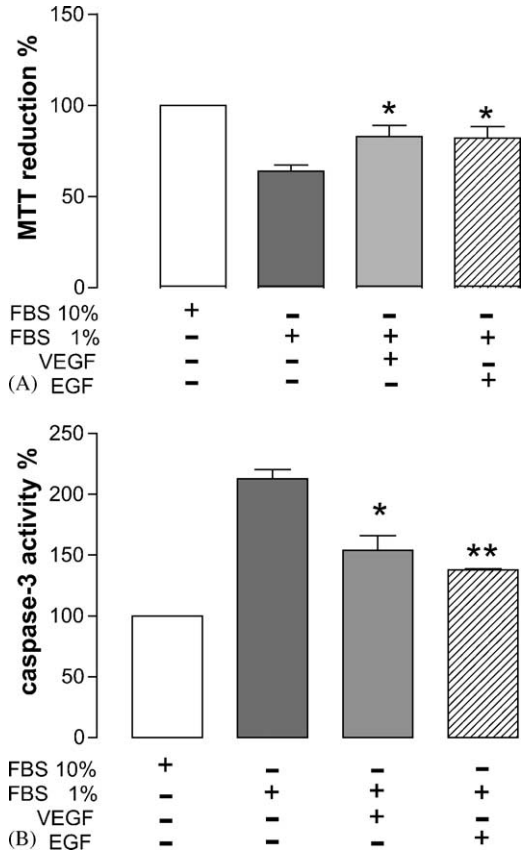

Fig. 1. Effect of VEGF and EGF on the apoptosis induced by serum deprivation in primary cultures of HUVEC. (A) HUVEC were plated at the density of 1×10^4 cells/well in 96-well plate. Immediately before the experiment the cells were washed twice with PBS and incubated in M199 containing either 10% (v/v) FBS or 1% FBS plus 1 mg/mL BSA; 50 ng/mL VEGF or 80 ng/mL EGF were added where indicated. After 18 hr MTT test was performed. Absorbance values were normalized to the control sample (FBS 10%) which was taken as 100%. Data are expressed as mean ± SE of four independent experiments made in sixtuplicate. (B) HUVEC were plated at the density of 63×10^3 cells/well in 24-well plate. Cells were treated as described in (A); after 3 hr incubation, the cells were harvested in lysis buffer for determination of caspase-3 activity. Fluorescence values were normalized to the control sample (FBS 10%) which was taken as 100%. Data are the mean ± SE of three independent experiments performed in duplicate. $^{**}P < 0.001$; $^{*}P < 0.05$.

As reported in several cells [14], including HUVEC [18,19], we found that staurosporine caused apoptosis (Fig. 2) when HUVEC were incubated in a serum-deficient

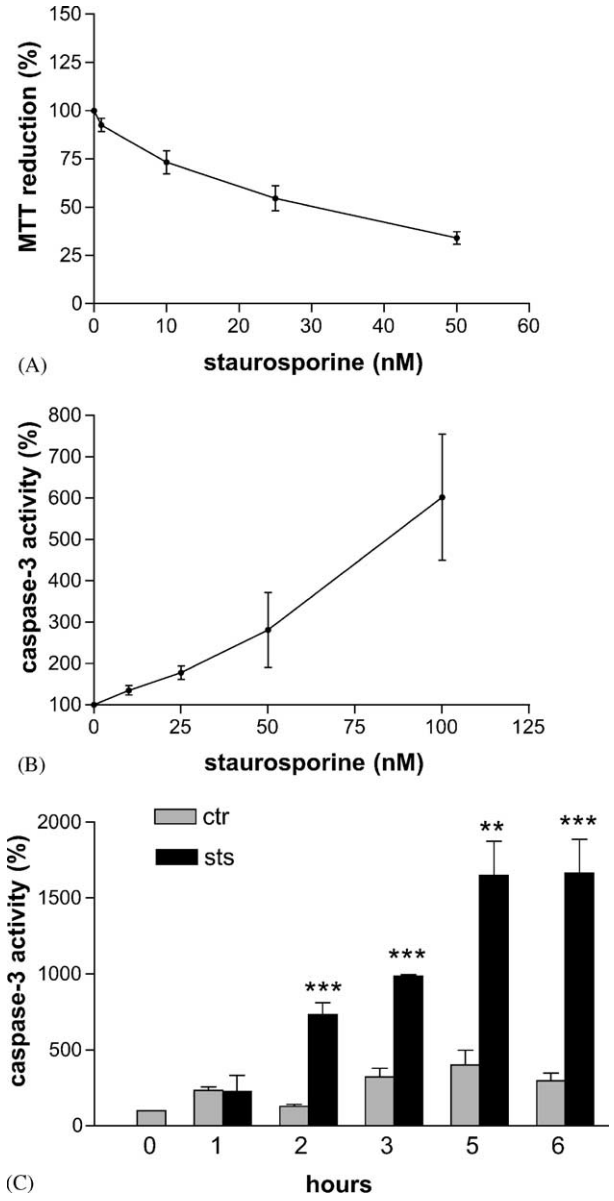

Fig. 2. Apoptotic effect of staurosporine in HUVEC. Cells were plated at a density of 35,000 cells/cm^2 and immediately before the experiment were incubated in serum-deficient medium (M199 with 1% FBS, 1 mg/mL BSA) containing various concentrations of staurosporine: (A) MTT reduction was determined after 18 hr. (B) Caspase-3 activity was determined after 3 hr. (C) HUVEC were incubated in serum-deficient medium in the presence or absence of 50 nM staurosporine (STS), caspase-3 activity was measured at the indicated time. Data are expressed as mean ± SE of six independent experiments made in sixtuplicate (A) and two independent experiments performed in duplicate (B, C). In (A) and (B) the value of the sample without staurosporine was taken as 100%. In (C) the value of the sample without staurosporine at time 0 was taken as 100%. Statistical analysis: linear regression of (A) and (B) curves gives P values of 0.0056 and 0.0015, respectively; (C) unpaired t-test comparing STS vs. control at the indicated time, $^{**}P < 0.01$ $^{***}P < 0.001$.

medium. The loss of cell viability at 18 hr of incubation was concentration-dependent, with an EC_{50} of 30 nM (Fig. 2A). The decrease of MTT reduction was preceded by a concentration-dependent increase of caspase-3 activity (Fig. 2B). As shown in Fig. 2C, the activation of caspase-3 in HUVEC treated with staurosporine started after 2 hr of incubation with the inhibitor and reached its maximum after 5 hr. The pro-apoptotic action of staurosporine was manifest at 10 and 1% (v/v) serum concentration in cell culture (Fig. 3). The presence of 50 ng/mL VEGF in the incubation medium during the treatment with staurosporine prevented the increase of caspase-3 activity at the two serum concentrations, indicating a protective effect. EGF (80 ng/mL) was also active. Separated experiments showed that the decrease of caspase-3 activity caused by the growth factors in the presence of staurosporine did not avoid the progressive loss of cell viability which slowly progressed in the samples containing staurosporine, reaching 100% after 48 hr. These results indicate that the cytotoxic action of staurosporine is the result of several cellular effects, only partially influenced by the growth factors.

To confirm that the VEGF-induced decrease in caspase-3 activity in the presence of staurosporine was an anti-apoptotic action we performed a TUNEL-POD assay, an *in situ* cell test that detects DNA fragmentation. It was observed that the addition of 50 nM staurosporine to the serum-deficient culture increased the number of TUNEL-positive cells after 3 hr of incubation. VEGF (50 ng/mL) decreased the number of TUNEL-positive cells in the absence and in the presence of staurosporine (data not shown).

Time-course studies indicate that apoptosis is preceded by a modification of the level of the Bcl-2 family proteins [20,21]. Since the staurosporine-induced apoptosis as well as the VEGF-induced protection were evident after 3 hr of incubation, we examined the level of Mcl-1, an anti-apoptotic protein of the Bcl-2 family characterized by its rapid inducibility [22]. Western blotting analysis showed that the treatment of HUVEC with 50 nM staurosporine for 3 hr induced a 37% decrease in Mcl-1 protein content (Fig. 4). This effect was reverted if VEGF (50 ng/mL) was added to the cell culture medium during the incubation with staurosporine.

It has been shown that VEGF and EGF cause their action through the activation of PI-3K and ERKs pathways [5,23]. Therefore, by the use of specific inhibitors, we sought to explore if these signalling proteins were involved in the protective effect of the two growth factors against the staurosporine-induced apoptosis. In agreement with this possibility, we observed that the protective effect of VEGF was significantly reduced by the addition of 100 nM wortmannin or 25 μM LY294002, two structurally unrelated inhibitors of the PI-3K [24] and by the addition of 25 μM PD098059 or 25 μM U0126 inhibitors of MEK [25,26] (Fig. 5A). Similar results were obtained with EGF (Fig. 5B). In control cells (incubated in a medium with 1% FBS) the addition of 100 nM wortmannin or 25 μM LY294002 alone produced a 2-fold increase in caspase-3 activity while the inhibition of MEK by 25 μM PD098059 and 25 μM U0126 did not influence the caspase-3 activity

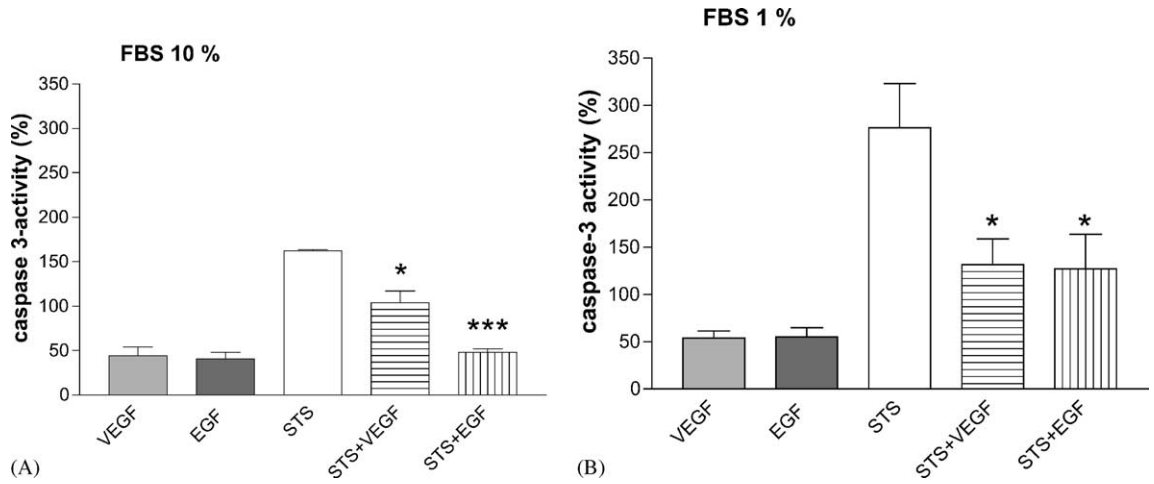

Fig. 3. Protective effect of VEGF and EGF on the apoptosis induced by staurosporine. HUVEC were plated at the density of 63×10^3 cells/well in 24-well plate. Immediately before the experiment the cells were washed twice with PBS and incubated in M199 containing either 10% (v/v) FBS or 1% FBS plus 1 mg/mL BSA; 50 ng/mL VEGF, 80 ng/mL EGF, 50 nM staurosporine (STS) were added where indicated. Caspase-3 activity was determined after 3 hr. Caspase-3 activity was expressed as percent of the activity measured in control sample (10 or 1% FBS). Data are the mean ± SE of three independent experiments performed in duplicate (A) or four independent experiments performed in duplicate (B). $^{*}P < 0.05$ STS + growth factors vs. STS (10 or 1% FBS), $^{***}P < 0.0001$ STS + EGF vs. STS (10% FBS).

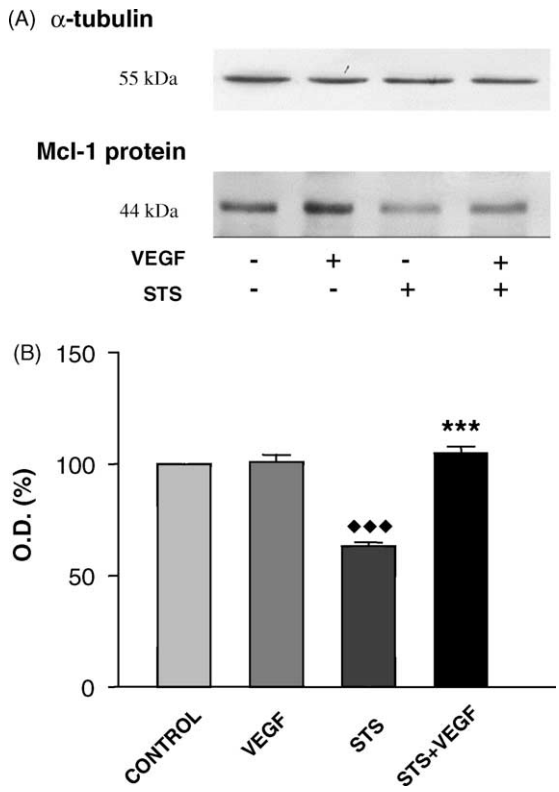

Fig. 4. Effect of VEGF on Mcl-1 protein level in HUVEC treated with staurosporine. HUVEC were plated in 100-mm dishes at the density of 2×10^6 cells/dish. Cells were incubated for 3 hr with serum-deficient medium (1% FBS) containing 50 ng/mL VEGF and 50 nM staurosporine (STS) as indicated. Cell lysates were processed for Western blotting analysis as described in Section 2. (A) Membranes were exposed to the primary antibody specific for human Mcl-1 protein; the same blot was stripped and reprobed with the α-tubulin antibody used as loading control. (B) Mcl-1 level was quantified by densitometry and OD values were normalized to the control sample that was taken as 100%; data are the mean ± SE of six independent experiments. $^{◆◆◆}P < 0.0001$ (STS vs. control), $^{***}P < 0.0001$ (STS + VEGF vs. STS).

(data not shown). This finding suggest that PI-3K activity also plays a role in maintaining the cell survival during serum deprivation.

4. Discussion

Since many years, it has been demonstrated that the programmed cell death is involved in the action of drugs. It has been shown that antineoplastic and immunosuppressant drugs such as methotrexate, etoposide, doxorubicin, methylseleninic acid and glucocorticoids cause apoptosis [11–13,27]. Following these investigations, we started a program aimed at establishing whether the iatrogenic apoptosis in HUVEC may be alleviated by the use of angiogenic growth factors. To this purpose we have selected the pro-apoptotic drug staurosporine and VEGF, a factor stimulating the proliferation and survival of endothelial cells [28]. The alkaloid staurosporine is extracted from streptomycetes and is used as a tool to inhibit protein kinase C (PKC) and other cell protein kinases. Subsequent investigations showed that this drug induces apoptosis in virtually all mammalian cells, including HUVEC [14,18,29]. The primary site of action of staurosporine in the apoptosis induction is not known but the effect has been dissociated from the inhibition of PKC [14]. Recently, it has been observed that staurosporine inhibits IκB kinase, thus preventing the synthesis of the anti-apoptotic proteins driven by NF-κB transcription factor. After this and other cellular effects, the action of staurosporine converges in the activation of the mitochondrial pathway of apoptosis with cytochrome *c* translocation and caspase activation [30]. However, alternative pathways are possible as staurosporine-induced apoptosis may also occur in cells defective in caspase activation [31].

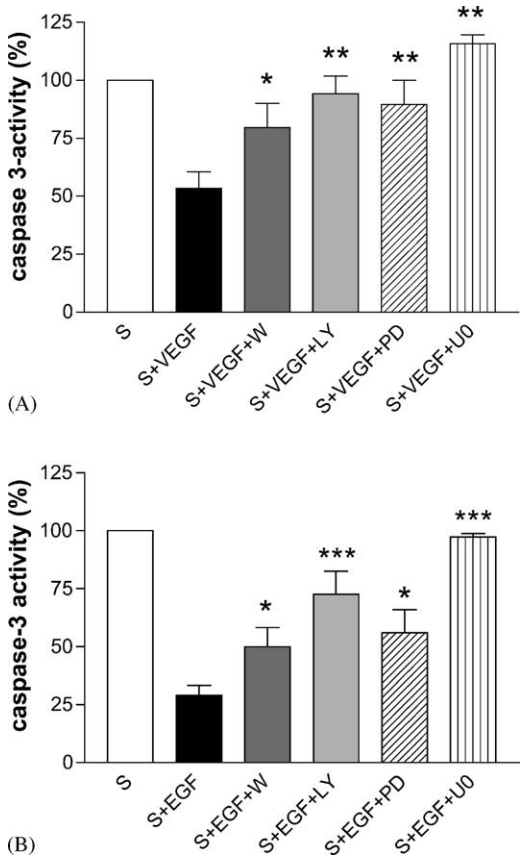

Fig. 5. Effect of PI-3K inhibitors and MEK inhibitors on the anti-apoptotic effect of VEGF (A) and EGF (B). HUVEC were plated at the density of 63×10^3 cells/well in 24-well plate. Cells were incubated in serum-deficient medium (1% FBS); 50 ng/mL VEGF, 80 ng/mL EGF, 50 nM staurosporine (S) were added as indicated. Where indicated a 30 min pre-treatment with 100 nM wortmannin (W), 25 μM LY294002 (LY), 25 μM PD098059 (PD) or 25 μM U0126 (U0) was performed before the addition of staurosporine and of the growth factors. The inhibitors were also included during the incubation with staurosporine and growth factors. Caspase-3 activity was determined after 3 hr and was expressed as percent of the activity measured in the cells treated with staurosporine alone. Data in (A) and (B) are the mean ± SE of at least three independent experiments performed in duplicate. Unpaired *t*-test was performed for the comparison of the effect of the inhibitors vs. S + growth factors: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

In agreement with these observations we found that the apoptosis elicited by staurosporine in HUVEC is associated with the activation of the pro-apoptotic caspase-3. This effect allows a rapid and reproducible procedure to study the combined action of staurosporine and its putative antagonists. Measuring the activation of caspase-3, we found that the staurosporine-induced apoptosis is a rapid process in HUVEC, being manifest after two hours of incubation and complete after 5 hr. In addition, our tests show that HUVEC are very sensitive to the action of staurosporine. At a low serum concentration (1%, v/v) the activity of staurosporine was detected between 10 and 100 nM. The same concentrations of staurosporine have been used in HUVEC by Fiorucci *et al.* [29] to test the antagonism by a nitric oxide-releasing acetylsalicilate derivative. These authors also showed that the action of staurosporine in HUVEC results in mitochondrial damage with progressive fall in membrane potential ($\Delta\psi_m$).

In agreement with our initial hypothesis, we found that VEGF added together with staurosporine, decreases the activation of caspase-3. Since the protection by VEGF led to a 24 hr delay in staurosporine-induced loss of cell viability, we conclude that the activation of the apoptotic pathways by this drug is delayed in the presence of VEGF. A further finding of our study is that EGF, a growth factor that is not specific for the endothelium, may duplicate the action of VEGF. Antagonism by EGF against the pro-apoptotic effect of staurosporine has been previously observed in two epithelial tumor cell lines, CNE-2 [32] and CE81T/VGH [15], however, the signal transduction pathway activated by EGF in the two cell lines seem to be different. In CNE-2, the anti-apoptotic action of EGF is mediated by PI-3K and PKC activation. On the other hand, the survival of CE81T/VGH cells is dependent on the activation of Raf-MEK-ERK pathway but not of PI-3K since wortmannin does not affect the protective effect of EGF.

It has been reported that cell survival signals induced by VEGF are mainly mediated by the PI-3K/Akt pathway [4,5,8]. Our experiments suggest that signals delivered by PI-3K pathway are important in maintaining cell survival also in serum-deficient cells. Pro-apoptotic effect of wortmannin and LY294002 has been reported earlier [8,32–34]. In HUVEC, it has been demonstrated that cell proliferation induced by VEGF and EGF is dependent on both PI-3K and ERKs, although the two growth factors act by different pathways [35]. Our data show that inhibition of PI-3K and MEK in staurosporine-treated cells prevents the anti-apoptotic action of VEGF and EGF suggesting that their protective effect requires the activity of both PI-3K and ERKs.

It is known that members of the Bcl-2 family of proteins can either promote cell survival (Bcl-2, Bcl-XL, A1, Mcl-1 and Bcl-W) or cell death (Bax, Bak, Bcl-XS, Bad, Bid, Bik, Bim, Hrk, Bok) (reviewed in [21]). The relative ratios of anti- and pro-apoptotic proteins determine the sensitivity and resistance of cells to a broad variety of cell death stimuli, including most anticancer drugs [19,36]. Cell survival mediated by VEGF and EGF have been correlated to their ability to modify the expression of Bcl-2 family proteins and other proteins involved in apoptosis [5,23]. Since the activation of caspase-3 induced by staurosporine was manifest after 2 hr of incubation and reached its maximum after 5 hr, we focused our attention in Mcl-1, an anti-apoptotic protein of the Bcl-2 family characterized by its rapid inducibility [22]. In agreement with the involvement of these regulatory proteins we observed that staurosporine decreases the protein level of Mcl-1 and that VEGF counteracts this effect, indicating that Mcl-1 could be involved in mediating the protective action of this growth factor. This is in agreement with the observation that Mcl-1 expression can delay or inhibit apoptosis

induced by staurosporine in CHO cells and in the CE81T/VGH cell line [15,37]. Furthermore, expression of Mcl-1 protein has been shown to be regulated by the activity of PI-3K [38–40], ERKs [15,22,41] or both [42].

In conclusion, we have shown that both VEGF and EGF protect HUVEC against the pro-apoptotic stimulus induced by staurosporine by signals transmitted through PI-3 K and ERKs. Further studies are required to ascertain whether the Mcl-1 expression in HUVEC is regulated by PI-3K or ERKs pathway.

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