PI3-KINASE AND MAP-KINASE INVOLVEMENT IN THE PROTECTIVE EFFECT OF VEGF ON STAUROSPORINE-INDUCED APOPTOSIS IN HUVEC

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Vascular endothelial growth factor (VEGF) is a potent angiogenic factor that mediates developmental, phisyological and pathological neovascularization. Previous studies have demonstred that VEGF prevents apoptosis and promotes survival of human dermal microvascular endothelial cells (HDMEC) and of human umbilical vein endothelial cells (HUVEC) in serum free colture (Gupta et al.1999, Gerber et al. 1998), moreover VEGF prevents ceramide-induced apoptosis (Gupta et al. 1999) and tumor necrosis factor (TNFα)-induced apoptosis (Spyridopoulos et al. 1997). It is not yet known whether VEGF inhibits the apoptotic cell death induced by pharmacological agents in HUVEC.
The aim of our study was to evaluate the protective effect of VEGF on staurosporine-induced apoptosis in HUVEC and to elucidate the molecular mechanism underlying this protective effect. Staurosporine, taken as a model of pharmacological agent inducing apoptosis, promotes apoptosis by the realese of cytochrome c. It has been shown that epithelial growth factor (EGF) can inhibit staurosporine-induced apoptosis in human oesophageal carcinoma cell line (Cheun Mun Leu et al. 2000). VEGF exerts its biological effects by binding to its respective transmembrane receptors, VEGF.receptor-1 (flt-1) and VEGF-receptor-2 (Flk-1/KDR) both of which are expressed on HUVEC specifically and contain a cytoplasmatic tyrosine kinase domain. Stimulation of endothelial cells demonstred that VEGF can trigger the activation of MAP-kinase (including ERK and P38 MAP-kinase), PI3-kinase, P70 56 kinase, PLCγ (Marshall, 1995; Sato et al. 1999) and focal adhesion kinase (Abedi and Zachary 1997). These data suggest that cytoprotective and mitogenic signals of VEGF might be transduced by independent pathways in endothelial cells.
We have shown that staurosporine induces apoptosis in a time and dose-dependent manner in HUVEC. The cell viability was tested following the reduction of MTT. The presence of apoptosis was evaluated by caspase-3 activity and by TUNEL-POD assay. Staurosporine at concentration of 50 nM caused loss of cell viability of 70% after 18 hours of incubation at 37° C, with respect to the control in serum free conditions. To confirm that the effect of staurosporine was independent of serum starvation, the effect of staurosporine on HUVEC was examined in the presence of 10% serum. After 3 hours staurosporine increased caspase-3 activity more than three times with respect to the control. The addition of 50ng/ml of VEGF or 80 ng/ml of EGF during the incubation with staurosporine delayed the loss of cell viability. Measuring the MTT reduction after 18 hours of incubation it was calculated that VEGF reduced the staurosporine-induced apoptosis of 26% ($n = 4$, $P< 0,005$) and EGF of 17% ($n = 4$, $P<0,005$). The protective effect of VEGF and EGF on staurosporine-induced apoptosis was confirmed in the caspase-3 activity test. In the presence of VEGF or EGF the caspase-3 activity was reduced after 3 hours more than three times compared to that of staurosporine alone (VEGF $n = 3$, $P<0,05$; EGF $n = 3$, $P<0,005$).
The action of VEGF and EGF was inhibited by 100nM of wortmannin and by 25uM of PD098059 suggesting a role of PI3-kinase and MEK/ERK pathways in the protective efect of VEGF and EGF on staurosporine-induced apoptosis. These data demonstrate a) the

occurence of pharmacological apoptosis in the human vascular endothelial cells, b) that VEGF and EGF in agreement with the observations on other cells (Cheun Mun Leu et al. 2000) considerably delay the progression of this apoptotic signal c) that PI3-kinase and MAP-kinase pathways are involved in the protective effect of VEGF and EGF.

References

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