HYBRID PROTEIN ENDOSTATIN-BAX PRESENTS HIGHER DEGREE OF APOPTOSIS THAN ENDOSTATIN AFTER ITS INTERNALIZATION BY ENDOTHELIAL CELL LINE. (C-PAE)

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Endostatin, a proteolytic fragment of collagen XVIII, is a potent inhibitor of angiogenesis and growth of various primary tumors. In the present study we have expressed a mutant of endostatin and compared its *in vitro* biological activity with the native endostatin. The mutant protein is composed of two functional domains: endostatin, that presents affinity for activated endothelial cells, used to guide the fusion protein and allow its internalization by the targeted cells, and a second domain composed by Bax, a short peptide of the pro-apoptotic protein Bax, corresponding to the minimal sequence required to promote apoptosis. In the mutant protein, the endostatin alpha helix was replaced by the alpha helix sequence of the Bax peptide. The mutant protein was obtained using site-specific mutagenesis and expressed in *E. coli* BL21(DE3) as insoluble cytoplasmic inclusion bodies. Western blotting assay confirmed its immunological identity. Endostatin as well as the hybrid were internalized, as shown by Western blotting. The degree of apoptosis was analyzed by flow citometry. C-PAE cells that were treated with the endostatin mutant exhibited a two fold higher degree of apoptosis if compared to the native endostatin (33,6 % and 14,5%, respectly). Our results indicate that the replacement of the endostatin alpha helix by the pro-apoptotic Bax peptide improves their apoptotic activity.

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