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A Synthetic Peptide that Corresponds to the C-terminal Region of HARP Inhibits Angiogenesis *in vivo* and *in vitro*

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Summary. Heparin Affin Regulatory Peptide (HARP), also known as pleiotrophin or heparin-binding growth-associated molecule, is an 18 kDa growth factor that has a high affinity for heparin. In the present work, we studied the effects of a synthetic peptide that corresponds to the last 25 amino-acids of the C-terminal region. In the in vivo chicken embryo chorioallantoic membrane model of angiogenesis, the peptide decreased the number of blood vessels in a dose-dependent manner. It also decreased the migration of human umbilical vein endothelial cells (HUVEC) in vitro, while it had no effect on HUVEC proliferation. Finally, the peptide also decreased the ability of HUVEC to form capillary-like networks when cultured on matrigel.

INTRODUCTION

Angiogenesis is a complex, multistage process responsible for the formation of new blood vessels from pre-existing ones and is essential for growth and metastasis of tumors. Endothelial cells play important roles in the regulation of the angiogenic process, during which they migrate, proliferate and differentiate to form new vessels (1). These processes are mediated by numerous natural mediators and pharmacological agents that act either as stimulators or inhibitors of angiogenesis. Heparin Affin Regulatory Peptide (HARP) seems to be significant among the natural mediators of angiogenesis (2-4).

HARP has an approximate molecular weight of 18 kDa when analyzed by SDS-PAGE. It consists of 168 amino acids, the first 30 corresponding to a signal peptide. The mature molecule is highly conserved among species (with an overall homology of 98% among human, rat and bovine) and is rich in basic amino acids (mainly lysines) forming two clusters at the termini of the molecule. HARP also contains 10 cysteines contributing to the formation of two β-sheet domains at the central region of the molecule. The two lysine-rich termini of HARP are also characteristic, each one forming an α-helix and containing a basic consensus sequence present in many heparin-binding growth factors (5). We have previously shown that human recombinant HARP expressed in bacteria was mitogenic for three endothelial cell types when it was presented to cells as a substrate (6). We have also shown that HARP is angiogenic in vivo, in the chicken embryo chorioallantoic membrane (CAM) assay and *in vitro*, using different types of endothelial cells (7).

In order to characterize domains of HARP with potential implications in angiogenesis, one peptide corresponding to the last 25 amino acids of the C-terminal region of HARP was synthesized

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and its effect was studied on several angiogenesis assays *in vivo* and *in vitro*.

METHODS

The sequence of the tested peptide was NH_2-KLTKPKQAESKKKKKEGKKQEKMLD-COOH. The peptide was synthesized by solid phase peptide synthesis techniques, on a 2-chlorotrityl-chloride resin, using Fmoc/t-butyl protecion strategies. The in vivo chicken embryo CAM angiogenesis model was used, as previously described (8). Human umbilical vein endothelial cells (HUVEC) were isolated from human umbilical cords and cultured as previously described (9). Migration and tube formation on matrigel were studied as previously described (7). The significance of variability between the results from each group and the corresponding control was determined by unpaired t-test. Each experiment included triplicate measurements for each condition tested, unless otherwise indicated. All results are expressed as mean ± S.E.M. from at least three different experiments.

RESULTS

The peptide inhibited angiogenesis in the CAM in a dose-dependent way, reaching the maximum inhibition at the dose of 1 $\mu g/cm^2$. The peptide had no effect on HUVEC proliferation whereas it inhibited HUVEC migration and tube formation on matrigel in a dose-dependent manner, reaching the maximum inhibition at the dose of 10 ng/ml.

CONCLUSIONS

- The peptide that corresponds to the COOH terminus of HARP inhibits angiogenesis in vivo and endothelial cell functions in vitro.
- Further studies are in progress in order to elucidate the mechanisms through which this peptide inhibits angiogenesis and to investigate its potential as an angiogenesis inhibitor.

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