Investigations of the effect of a cyclic RGD peptidomimetic on cell proliferation, migration and angiogenic activity in human endothelial cells

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Background: Angiogenesis is a physiological event occurring in the development of organisms, wound healing and the reproductive cycle and involved in pathologic processes such as inflammation, tumour growth and metastasis [1]. Among the proteins involved in this process, integrins play an important role by promoting endothelial cell attachment and migration on the surrounding extracellular matrix, cell to cell interaction and intracellular signal transduction [2]. Several integrins, including α_V, $\alpha_5\beta_1$ and $\alpha_{IIb}\beta_3$, recognize Arg-Gly-Asp (RGD) sequences in endogenous ligands [3]. A new class of cyclic RGD peptidomimetics, containing a bifunctional diketopiperazine scaffold, show a low nanomolar affinity for integrins $\alpha_V\beta_3$ and $\alpha_V\beta_5$ [4,5]. The present study is aimed to characterize the ability of *cyclo*[DKP-RGD] **1** (RGD1) to affect viability, proliferation, migration and capillary network formation in human umbilical vein endothelial cells (HUVEC).

Methods: HUVEC were cultured under standard conditions in basal medium (BM) or with the addition of rh-VEGF, rh-EGF, basic rh-FGF, rh-IGF-1(5 ng/ml) and 10% FBS as activating stimuli. Cell viability tests were performed by means of flow cytometry and propidium iodide (PI) staining. Proliferation was measured by an ELISA assay based on measurement of bromodeoxyuridine incorporation during DNA synthesis. Cell migration was measured by means of a Boyden chamber assay. For the angiogenic assay, HUVEC were seeded on plates coated with Matrigel and tube?like structure formation was evaluated by phase?contrast microscopy. Data are shown as means±standard deviation (SD). Statistical significance of the differences was assessed by two-tailed Student's *t* test for paired data.

Results: HUVEC viability at 24 h was 70±4% in basal conditions and 80±11% in stimulated conditions ($P>0.05$). Cells proliferation in basal conditions was 0.3±0.2 OD and increased up to 1.9±0.5 O.D. in stimulated conditions ($P<0.001$). RGD1 (0.1 nM – 10 μM) did not affect either viability or proliferation.

Spontaneous migration of HUVEC in basal conditions was 32.4±8.9 μm and increased up to 54.3±11.6 μm in stimulated conditions ($P<0.0001$). RGD1 increased both spontaneous and stimulated migration in a concentration-dependent manner showing a bell shaped response curve. Maximum migration occurred with 1 nM RGD1, which increased both basal and stimulated migration by 112±88% and 28±22% ($P<0.001$ and $P<0.01$ respectively).

HUVEC under basal conditions did not show tube-like structure formation while incubation in stimulated conditions induced a significant tube growth that was mimicked by incubation with 1 nM IL-8. RGD1 did not affect tube-like structure formation in HUVEC under basal conditions while addition of RGD1 to stimulated cells significantly decreased tube growth. Maximum inhibitory effect was exerted by 1 μM RGD, which decreased tube growth 39±13% with respect to values measured in stimulated conditions ($P<0.001$) and by 63±8% the presence of IL-8 ($P<0.001$ *vs* IL-8 alone).

Conclusion: The data of the present study show that the novel compound RGD effectively inhibits angiogenic processes in HUVEC without affecting their viability and proliferation. This compound may be therefore a candidate modulator of angiogenesis, possibly devoid of the adverse effects of structural analogues, such as cilengitide, which also exert significant cytotoxic effects. Further studies clarifying the *in vivo* activity of RGD, including a complete toxicological assessment, as well as a thorough investigation of the intracellular pathways involved its effects are currently underway in order to evaluate its therapeutic potential.

References

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