B9 - Human Recombinant Oligomeric Endostatin for Inhibition and Regression of Pathological Angiogenesis

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Introduction:

Endostatin is a potent endogenous angiogenesis inhibitor, fully suppressing and regressing tumor growth in mouse models, showing no signs of toxicity and lack of resistance on cyclic treatment. In clinical trials, however, tumor regression was not achieved. Crystallographic studies have shown that endostatin binds a zinc ion through histidine coordination at is N-terminal region possibly forming dimers, although they were never observed in solution. Artificially induced endostatin dimers, but not monomers, have been shown to promote endothelial tubes disassembly in vitro, which suggests modulation of antiangiogenic activity by oligomerization.

Objective:

Investigate zinc-induced structural modifications in endostatin that may correlate to its biological function.

Methodology:

Molecular dynamics computer simulations were performed with endostatin (PDB 1BNL) using GROMACS software package and GROMOS96 force field. Cut-off radius for non-bonded interactions was set to 1.8 nm and 1.2 nm for Coulomb and Lennard-Jones potentials, respectively. Recombinant human endostatin was expressed in P. pastoris in two different conditions of controlled pH (pH 6.0 and 7.5). Size exclusion chromatography was performed using GPC 100 HPLC column. Biological activity in vitro was accessed by Matrigel tube regression assays.

Results:
Molecular dynamics simulations performed in the absence of zinc showed significant unfolding of the Nterminal loop of endostatin, leading to dimer dissociation. When zinc was present, on the other hand, dimers were stable through the entire time of the simulation. When recombinant human endostatin was expressed at controlled pH in Pichia pastoris, endostatin dimers were observed in solution at pH 7.5, while no dimer formation was detected in endostatin preparation at pH 6.0. Endostatin produced at pH 7.5 has biological activity in Matrigel tube regression assay in nanomolar range, whereas the preparation at pH 6.0 and the clinical formulation (endostatin in citrate buffer, pH 6.2) were inactive.

Conclusion:

The patent application PI0605212-6, here presented, is related to a preparation of oligomerizable endostatin, characterized by the non-covalent association between monomers, presented in a soluble form and compatible with clinical administration. In order to obtain tumor regression with antiangiogenic therapy, stopping tumor vasculature from growing may not be enough; it needs also to be disassembled. Abundant evidence in the literature demonstrates that monomeric endostatin can inhibit angiogenesis by blocking de novo formation of capillaries. On the other hand, disassembly of pre-established tubes was reported exclusively by artificially oligomerized endostatin. Based on these data, we propose that the lack of activity in clinical trials could be due to the fact that the endostatin produced for human therapy was exclusively monomeric, produced and administered at acidic pH. Our work shows that producing recombinant human endostatin in P. pastoris at a pH compatible with zinc coordination by histidines preserves the dimeric structure of endostatin and its full biological activity, perhaps leading to a better therapeutic outcome.

Keywords: endostatin, human recombinant oligomeric, angiogenesis, cancer