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Development and evaluation of a new type of wound healing stimulators based on binding loops of the PDGF and VEGF growth factor families

Annually about 1-2% of Europeans suffer from chronic wounds, the treatment of which consumes up to 2-3% healthcare budget. Particularly problematic groups are patients after radio- and chemotherapy, people with chronic bedsores, venous ulceration, and importantly patients with diabetes. The data of the last one show that about 15-30% of patients struggle with the problem of non-healing wounds leading to diabetic foot ultras. This type of wound, in addition to the high cost of treatment, leads to amputation of the limbs in about 20% of cases. The pathophysiological basis is still far from being known. However, many studies indicate that a decrease in the level of growth factors may be the lead. When skin is injured, the growth factors are produced to bind to their extracellular receptors on the cells and force them to proliferate, migrate, and differentiate. Studies show that patients with diabetic foot ultras express a lower level of growth factors (and their receptors) such as platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF). Both factors exist as hetero or homodimers like PDGF-BB, AB, CC, DD, and VEGF-AA, BB, CC, DD, etc. They bind to receptors such as PDGFR α/β, and VEGFR1-2, which cause their dimerization and activation by intracellular phosphorylation. The genes encoding PDGF and VEGF proteins show many structural and functional similarities by sharing conserved sequences across the family. Recent studies indicate that PDGFs bind not only to their receptors but also VEGFR1/2 receptors, with high affinity. Studies by our group, among others, have shown that not only a short fragment of the PDGF-B binding loop is enough to bind the PDGF α/β, and andVEGFR2 receptors, but also to accelerate wound epithelization in the mouse model. However, despite their interesting effects, these peptides are not as effective as native PDGF-BB protein.

Therefore, based on the structural, sequence, and similarities in the mechanism of growth factors activation by receptor dimerization, our goal is to obtain new compounds with dimer structures that mimic the natural mechanism of the action. For this purpose, we will design and synthesis, a pool of compounds based on the binding loops of PDGF-A/B/C/D and VEGF-A/B/C/D. To rationalize the design we will compare the structure/sequence of different growth factors among PDGF and VEGF, and employ molecular modeling. The available crystal structures of complexes PDGFβ:PDGF-BB, and VEGF1:VEGF-AA will be utilized. Subsequently, a pool of new compounds will be screened for their binding affinity to PDGFRα,β, and VEGFR1,2 receptors (ELISA assays) and their stimulatory potential will be assessed on the skin cell cultures (fibroblasts/keratinocytes). Next, the most promising peptides will be subjected to a human serum stability study, and the structure of a few of them will be determined by NMR (and CD) spectroscopy. Collected data will be used to redesign new peptides and peptidomimetics based on molecular modeling, in coupling with biological results. The new pool of compounds will be screened again (ELISA, cell lines), and the affinity of several of them to receptors determined by the MST technique. The most promising compounds will be obtained in the form of homo- and heterodimers (also in the system, e.g. PDGF-B/VEGF-A) linked to each other by PEG linkers in a "click chemistry" type reaction. The stimulatory potential of the dimers will be determined on cell lines (proliferation, migration, toxicity, chemotaxis) and on primary lines isolated from healthy and diabetic patients. The primary lines will also be screened for PDGF and VEGF receptor expression to correlate their presence with compound activity in cell culture. In addition, selected dimers will be screened for their potency to activate VEGF and PDGF receptor phosphorylation and major receptor-related pathways (MAPK, ERK1/2, etc.) by western blot, and ELISA techniques.

The result of the project will be the verification of whether a new type of potential wound healing stimulators based on the dimerization-type of action will be able to mimic the natural mechanism of growth factor receptor activation. This unique type of approach in the design for wound healing stimulators is not yet described in the literature. Verification of this model will be extremely valuable and may be used for the design of next-generation drugs.