Preclinical characterization of drug like glucocorticoid induced leucine zipper peptide analogs.

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Many intermolecular interactions in a eukaryotic cell are mediated through protein-peptide interactions. For efficient interaction, the peptide scans the protein surface for a large enough pocket into which it anchors through a small number of residues/core motif that contribute maximally to the free energy of binding. Of special significance is the preponderance of proline rich sequences that preferentially adopt the left-handed polyproline type II (PPII) helical conformation in the interface peptides. Availability of both side chain and backbone carbonyls for interaction makes PPII helix an excellent recognition motif. Glucocorticoid induced leucine zipper (GILZ), is a glucocorticoid responsive protein that has been shown to suppress immuno-inflammatory responses by preventing the nuclear translocation of the p65 subunit of the transcription factor nuclear factor-kappa B (NF-κB). Mutational and binding studies localized the sites of interaction to the proline rich region at the carboxy terminus of GILZ and the transactivation domain of p65. Similar to most intermolecular interactions mediated by proline rich motifs the strength of interaction between the GILZ and the p65 proteins is in the micromolar concentration suggesting weak binding kinetics. A widely used strategy in the discovery of peptide drugs involves exploitation of the complementary surfaces of the naturally occurring binding partners. We observed that a synthetic peptide (GILZ-P) derived from the proline rich region of GILZ suppressed immune mediated inflammatory responses in mice. Here we characterize GILZ-P structurally and evaluate its toxicity and efficacy in mature human macrophage like THP-1 cells. We show that the GILZ-P adopts an extended polyproline type II helical conformation. Functionally GILZ-P is non-toxic, suppresses NF-κB activation by activated macrophages suggesting a therapeutic potential in pathologies wherein persistent inflammation plays critical role in the disease initiation and/or progression.