CHARACTERIZATION OF A MULTIPLE TARGET KINASE INHIBITOR USING DIFFERENT INFLAMMATORY CELLULAR MODELS

Attila Varga[1] Pál Gyulavári[1], Zoltán Greff[2], Tamás Németh[3], Krisztina Kerekes[4], Diána Brauswetter[5], Márton Kokas[5], Anna Erdei[4], Attila Mócsai[3], György Kéri[1,2,5], Tibor Vántus[5]

[1] MTA-SE Pathobiochemistry Research Group, Budapest, Hungary
[2] Vichem Chemie Research Ltd., Budapest, Hungary
[3] Department of Physiology, Semmelweis University, Budapest, Hungary
[4] Department of Immunology, Eötvös Loránd University, Budapest, Hungary
[5] Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, Budapest, Hungary

Background: Inflammatory diseases have very severe effects on healthcare worldwide, therefore development of novel inflammatory pathway specific inhibitors would be a great value. Amongst many kinases, vascular endothelial growth factor receptor 2 (VEGFR2) and protein kinase D1 (PKD1) are potential targets in inflammatory diseases. Since, in the last few years the paradigm of drug discovery changed from the single target drug to the multiple target drug approach, our aim was to identify and characterize a multiple target kinase inhibitor which can efficiently inhibit VEGFR2 and PKD1 related cell functions in inflammatory cell models.
Methods: We used the immobilized metal affinity for phosphocemicals (IMAP) method for the recombinant VEGFR2 and PKD1 kinase assays. In the endothelial EA.hy926 cell model we used western blot analysis to investigate the intracellular effect of the inhibitor, furthermore wound healing and Matrigel based tube formation assays to explore the anti-angiogenic effect of the compound. Immune-comlpex induced superoxide production of human neutrophils was determined by ferricytochrome c assay. Mediator secretion by RBL-2H3 mast cells in response to stimulation by FcεRI clustering was monitored by measuring activity of the secreted granular enzyme β-hexosaminidase.
Results: After the screening of a kinase specific molecule library, we selected the best VEGFR2 and PKD1 inhibitor and determined its biochemical IC_{50} value. Then, we tested the effect of this inhibitor on different inflammatory cell based models. Our first model was the EA.hy926 endothelial cell line, where our inhibitor effectively diminished endogenous VEGFR2 and PKD1 activation, furthermore reduced VEGFR2 and PKD1 involved cellular functions, such as cell migration and *in vitro* angiogenesis. In human neutrophils PKD1 is involved in immune-complex induced superoxide production, which was reduced by our inhibitor in a concentration dependent manner. Furthermore, in mast cells PKD1 is implicated in FcεRI triggered mediator release, which was also attenuated by our compound.
Conclusion: We have identified and characterized a multiple target kinase inhibitor, which could effectively block VEGFR2 and PKD1 related cellular functions in different inflammatory cell models.

Poster presentation
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