TARGETIBDBYPLA2 Report Summary

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Final Report Summary - TARGETIBDBYPLA2 (A Novel Drug Targeting Strategy for the Treatment of Inflammatory Bowel Disease: A Molecular Biopharmaceutical Approach)

The overall goal of this research was to mechanistically enable specific targeting of drugs to the diseased tissues in IBD, by a novel PL-drug conjugate, from which the drug is released at the target site(s) by PLA2.

First, we synthesized different PL carriers. We reacted LPC in four reactions, with fmoc-6-AHA, fmoc-8-AoCoH, fmoc-GABA, and fmoc-Gly. The reactions were made in dichloromethane with the assistance of EDCI and DMAP. Afterwards we checked our samples with thin layer chromatography (TLC) to ensure reaction has carried out. The separation was made by flow chromatography. After obtaining positive results with 6-AHA we carried on to the second step – attaching 6-AHA (the linker) to Diclofenac (the drug). Diclofenac was added by drop-wise technique to minimize the number of products we get from the reaction. Work-up and separation steps are the same as in the first reaction. Overall, we have completed the synthesis and full characterization of 4 PL-Drug conjugates: Diclofenac attached to the sn-2 position of a PL through 4 different linkers, 2-, 4-, 6- and 8-carbon linkers.

After obtaining the conjugates, we moved to the in-vitro characterization of the prodrugs activation by PLA2. The in-vitro results indicated that the 6-carbon and the 8-carbon linker lengths were highly activated by PLA2, while the recognition between the PLA2 enzyme and conjugates with shorter linkers was insufficient. These results are in corroboration with our previously published data for indomethacin-PL conjugates.

Next, we established an IBD model, using modern powerful in-silico method. In summary, we developed a molecular modeling procedure to estimate relative activity of PLA2 enzymes toward lipid prodrug molecules. In a nutshell, Molecular Dynamics simulations of transition state complex of the prodrug/PLA2 in water, followed by geometry optimization of the complex as well as geometry optimization of the isolated prodrug, was performed. Then, prodrug deformation energies in the catalytic transition state complex with PLA2 were calculated. As the model validation method, we correlated the computed results with published experimental data. Simulations were run with both human and bee venom PLA2, to estimate the relevance of experimental results from bee venom PLA2 to humans. This method permits to optimize the chemical structure of the linker connecting the drug moiety to the lipid and reduce the amount of chemical syntheses needed in order to develop effective prodrugs. The method correlated very well with both our in-vitro data obtained in this project (diclofenac-PL) as well as with our previously published experimental data (indomethacin-PL). Predicted optimum length of the linkers for lipid prodrug molecules based on indomethacin, diclofenac and methotrexate are 5-6 CH2 units while for tacrolimus prodrugs it is slightly higher at 6-7 CH2 units.

From a clinical point of view, the potential to significantly improve our treatment options in IBD, especially in CD: current IBD drug targeting mechanisms rely on pH, time, pressure or microflora changes along the GIT. These mechanisms may be effective in targeting the colon, due to the significantly different environment compared to the small intestine, but no mechanism targets the drug to the sites of inflammation localized in the small intestine, and hence all currently available drugs fail to be effective for CD patients. The main potential impact is, therefore, achieving drug targeting to the inflamed mucosa per se, regardless of the localization of the disease. Since significant overexpression of PLA2 has been shown in both UC and CD, irrespective of the disease localization, and since PLA2 is the enzyme activating our PL-Drug conjugates and liberating the free drug, the developed novel prodrugs are expected to effectively target the drug to the diseased intestine in any IBD patient – CD or UC – regardless of the disease location. If successful, this drug targeting strategy will make a
significant contribution to drug therapy and management in IBD patients. Not only will the proposed research will improve treatment options in CD, but our management of UC patients may also be significantly improved by the proposed prodrug approach. The colonic lesions of UC patients may be distributed in different regions throughout the colon, e.g. proctitis, left-sided colitis, total colitis, and complications such as toxic megacolon and backwash ileitis. Each of these very different clinical presentations has different needs with respect to drug targeting. These needs are, however, not being met today, even though all these conditions can be treated with an identical approach, such as the present research. This novel approach will allow a single drug product to target the diseased intestine per se and hence to deliver the drug to the region where it is really needed, thereby significantly improving management of IBD – both CD and UC – patients.

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