Drug-target residence time : a case for the adenosine A1 and A2A receptors

Guo, D.

Summary

The present thesis starts with a general description of the current drug research pipeline and the high attrition rate therein (Chapter 1). Lack of clinical efficacy was reported as the main cause for drug failure. The concept drug-target residence time was then introduced, which is a paradigm suggested to be a better predictor for drug efficacy than the equilibrium–based metrics (i.e., K_i or IC_{50}). The adenosine A_1 and A_{2A} receptors were chosen as two prototypical GPCRs for this thesis, since many ligands have been developed for these receptors after decades of medicinal chemistry efforts. Those compounds were good starting points for investigating ligand-receptor binding kinetics.

In Chapter 2, a series of prototypic GPCR ligands that have known kinetic information is reviewed. This is followed by an extensive discussion of the kinetic behaviour of GPCR ligands at four representatives GPCRs, which are the muscarinic M_3, tachykinin NK_1, dopamine D_2 and corticotropin-releasing CRF_1 receptors. It was found that there is much less kinetic data for GPCR ligands than equilibrium-based data determined in the early phases of drug discovery; almost no detailed structure-kinetics relationship (SKR) studies have been reported previously, if any.

Chapter 3 contains the set-up, validation and optimization of a novel kinetic screening method, the so-called dual-point competition association assay. This method enables fast and high-throughput screening, since it only requires a measurement of radioligand binding at two different time points. Such a dual-point measurement yields the kinetic rate index (KRI), a parameter coined for quickly assessing kinetic parameters. In this chapter, a screen with 35 high affinity A_1R antagonists was described. Seven compounds were identified with a KRI-value above 1.2, indicating a relatively slow dissociation from the target. All other compounds had a KRI-value below or equal to 1.0, predicting a relatively fast dissociation rate. Several of these compounds were selected for follow-up kinetic quantification and were shown to have off-rates that corresponded to their KRI-values. Thus, the KRI and the dual-point assay can robustly predict a competitor's dissociation rate. This assay can be applied to a wide range of drug targets as well.

In Chapter 4, we performed an extensive SKR study on the $A_{2A}R$ in addition to a traditional SAR analysis—a combination strategy proposed in Chapter 2. The ensemble of 24 $A_{2A}R$ antagonists, all ZM241385 derivatives, displayed only minor or insignificant changes in affinity, while they varied substantially in their dissociation rates from the receptor. We believe such a combination of SKR and SAR analysis as on the $A_{2A}R$ will have general importance for other drug targets as well, since it can serve as a new strategy to tailor the interaction between a ligand and its receptor.

An exploration of the molecular basis behind ligand (*in vitro*) efficacy is described in Chapter 5. In this chapter the binding kinetics of ten $A_{2A}R$ agonists from different chemical classes were determined and the putative relationship to their functional efficacies at the $hA_{2A}R$ was extensively examined in Chapter 5. Agonist binding kinetics were determined using the competition association method. The $A_{2A}R$ agonist funtional efficacies were measured in a label-free impedance-based assay and in a cAMP assay. It was found that the ligands' efficacy at the adenosine A_{2A} receptor is correlated with their divergent target-residences times.

Likewise, the investigation of A_1R allosterism from a binding kinetics perspective helped us in further understanding the molecular basis of receptor allosterism (Chapter 6). We showed that an allosteric modulator can alter the orthosteric ligand's dissociation rate as well as the association rate; the latter less investigated before in literature. Such influence was probe- and concentration-dependent. This chapter also contains an examination of the binding kinetics of two bitopic ligands, LUF6234 and LUF6258, which consist of an allosteric and an orthosteric pharmacophore connected via a linker. Covalently linking both parts into one molecule had a substantial effect on the overall on- and off-rate. These (mathematical) analyses in terms of binding kinetics can add a wealth of knowledge to current pharmacological receptor concepts.

Finally, general conclusions from the research described in this thesis are given and future perspectives in this field are presented (Chapter 7). In short, this thesis provides insights in drug-target binding kinetics. We hope this thesis will bring more awareness of the importance of ligand-receptor binding kinetics and thus result in more kinetics-based studies in future early phases of drug design and discovery, eventually leading to different or even better-in-class drug candidates for future clinical applications.