Utility of a mixed effects approach to defining target binding rate constants

Neil Benson [1], Nelleke Snelder [2], Bart Ploeger [2], Carolyn Napier [1]
Harriet Sale [1] and Piet van der Graaf [1]

1. Pfizer Global Research & Development, Sandwich. Kent. CT13 9NJ, UK.
2. LAP&P consultants, Leiden, The Netherlands

Contacts: N.Benson@pfizer.com; N.Snelder@lapp.nl

Leiden Experts on Advanced Pharmacokinetics & Pharmacodynamics

Introduction

- Incorporation of target binding kinetics into PK/PD models has long been established (1).
- In recent years, there has been a marked increase in interest in designing 'slow-offset' ligands as a deliberate medicinal chemistry strategy to improve duration of action(2).
- Methodologies and principles in this area have been developing for almost half a century (3 and 4). However, defining transient target on-and off-rate kinetics remains a very resource intensive and reagent consuming process, with efficient parameter estimation often proving difficult in practice.
- In an attempt to address these issues, we have implemented kinetic models in NONMEM. We have applied these models to analyse simulated datasets and to optimize the experimental design for efficient identification of the transient target on- and off-rate kinetics. Finally, the optimal model was evaluated using real data. This analysis is an extension of the work of Karlsson *et al*, who stated that a simultaneous fitting procedure is superior to a separate fitting method for estimation of ligand binding parameters (5).

Objectives

- To quantify and qualify transient target on- and off-rate kinetics using NONMEM
- To optimize the experimental design: find the minimum of experimental work required for identification of the receptor dissociation and association rate constants

Methods

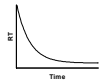
- A stepwise approach was followed:
  1. Identification of NONMEM model to describe simulated data from Gepasi (v3.30)
  2. Optimal design simulations
  3. Validation of the optimal design on real data
- Modelling was performed with NONMEM V.
- For simulations NONMEM V and VI were compared.
- The FOCE method with interaction was used.

Experiments

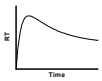
Exp 1: Measure the RT complex at equilibrium for several concentrations for identification of KdT

Exp 2: Following the time course of formation of the RT complex yielding information on $k_{on}T$

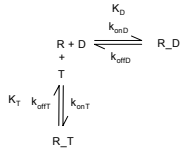
Exp 3: Evaluation of the time course of the dissociation of the RT (simulation) or RD (fitting) complex for information on $k_{off}T$ or $k_{off}D$



Exp 4: Following the time course of the RT complex after mixture of drug in 3 concentrations, tracer and receptor for identification of $k_{on}D$ and $k_{off}D$



Binding kinetics



KdD (Kd drug)=$k_{off}D/k_{on}D$
KdT (Kd tracer)= =$k_{off}T/k_{on}T$
R: target
D: drug
T: tracer
RD: target-drug complex
RT: target-tracer complex

Model

The following set of differential equations can describe the simulated data (figure 1):

$d[RT]/dt = k_{on}T*[R]*[T] - k_{off}T*[RT]$
$d[R]/dt = -(k_{on}T*[T]+k_{on}D*[D])*[R] + k_{off}T*[RT] + k_{off}D*[RD]$
$d[RD]/dt = k_{on}D*[R]*[D] - k_{off}D*[RD]$

IIV on Bmax, Proportional residual error
Assumption: [T] and [D] are in excess of [R]

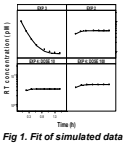


Fig 1. Fit of simulated data

Optimal experimental designs – by simulation

- Simulations to optimize the experimental design:
  1. Simulate data from all 4 experiments (100 replicates)
  2. Create new datasets by omitting one or more of the experiments
  3. Fit model to reduced datasets
  4. Calculate bias and precision
  5. Select the scenario with the smallest number of experiments with acceptable bias (<30%) and precision (<50%)

23% variability on Bmax and 22% residual error was introduced

Experiments 1 and 4 together yields sufficient information for identification of the association and dissociation rate constants as bias and precision were acceptable

NONMEM VI resulted in more accurate parameter estimates compared to NONMEM V

Validation of the optimal design on real data

Steps for model validation:

1. Fit experiments 1 & 4
   - parameters can be identified with acceptable uncertainty (figure 2 & 3 and table 1)
2. Fit experiments 1, 3 & 4
   - adding data from experiment 3 does not result in different parameter estimates or reduction of uncertainty (table 1)

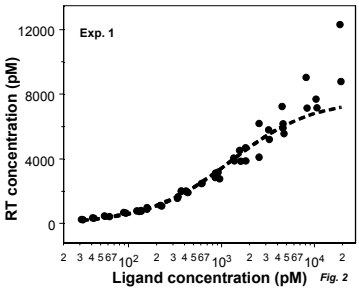


Fig. 2

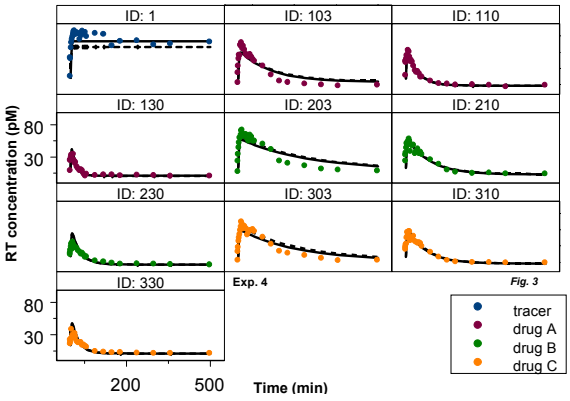


Fig. 3

Table 1. **Parameter estimates**

Parameter	Estimate without exp 3 (CV%)	Estimate with exp 3 (CV %)
$k_{off}T$ (1/min)	0.219 (18.5)	0.228 (13.6)
KdT (pM)	1170 (22.2)	1240 916.4)
$k_{off}D$ A (1/min)	0.00117 (7.5)	0.0014 (13.0)
KdD A (pM)	208 (28.5)	256 (22.5)
$k_{off}D$ B(1/min)	0.00068 (10.3)	0.00068 (9.7)
KdD B (pM)	261 (19.4)	271 (15.4)
$k_{off}D$ C(1/min)	0.000638 (7.3)	0.00064 (7.3)
KdD C (pM)	201 (16.9)	209 (12.5)
ω^2 Bmax (IIV)	0.00387 (79.8)	0.0033 (71.7)
σ^2 prop	0.0478 (19.0)	0.051 (17.5)

Bmax was estimated per study and is not reported

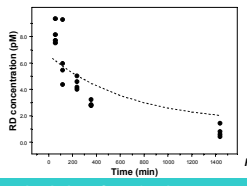


Fig. 4

Fig 2 & 3. Fits from exp. 1 and 4. Individual (solid) and population (dashed) predicted RT/D concentrations

Fig 4. simulation of exp 3 using parameters from exp 1 & 4. observations (dots) and prediction (dashed line)

Conclusions and perspectives

- A non-linear mixed effect modelling approach was applied for identification of ligand dissociation and association rate constants
- With data from the equilibrium exp. (1) and the exp. mixing receptor, tracer and drug (4) the association and dissociation rates could adequately be identified using real data
- This methodology allows the integration of target binding kinetics in drug discovery programs and is generically applicable to *in vitro* transient kinetics (eg. Enzymes, receptors, ion channels)

References

[1] Shimada *et al*, Biol. Pharm. Bull., 19(3), 430-437, (1996)
[2] Copeland, RA *et al*, Nat Rev Drug Discov. 2006 Sep;5(9):730-9. Epub 2006 Aug 4.
[3] Paton, WDM, Proc. R. Soc. Lond. B., 154, 21-69 (1961)
[4] Motulsky and Mahan, Molecular Pharmacology, 25:1-9 (1983)
[5] Karlsson and Neil, Mol. Pharmacol. , 35(1):59-66 (1989)