Nonlinear Pharmacokinetic Model For Interleukin-12 Gene Therapy

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Background: Several animal and human clinical studies have shown the therapeutic potential of interleukin-12 (IL-12) for the treatment of cancer and chronic viral hepatitis, although an down-regulation of the levels of IL-12, mediated by interferon γ (IFNγ), was observed in long-term treatments. Pharmacokinetic modelling is a useful tool to understand the mechanism of the different biological processes involved in the therapeutic response.

Objective: To develop a pharmacokinetic model that describes the behaviour of IL-12 and IFNγ at different doses in mice.

Methodology

I. Animal experimentation

Two groups of wild type (8 and 10 mice) and knock-out mice for the IFNγ receptor (7 and 9 mice) were infected with tw7 doses of a viral vector codifying for the interleukin gene ($2x10^8$ and $5x10^8$ iu). 14 days after the infection, interleukin expression was induced during 10 days by daily administration of mifepristone (RU), as is shown in figure 1. Levels of IL-12 and IFNγ were measured 24 hours before the firs induction and 10 hours after each daily injection for the lower dose. Regarding the higher dose, only levels of IL-12 were measured.

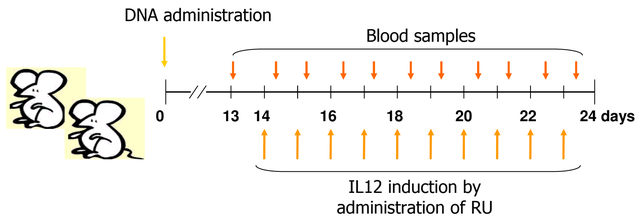


Figure 1. Scheme of the experimental design of the study

II. Pharmacokinetic model

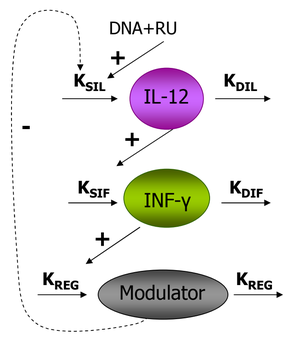
Three different pharmacokinetic models (Figure 2) were developed to describe the kinetic of IL-12 and the negative feedback trigger by the IFNγ in the wild type mice. Dose $2x10^8$iu was considered as DNA=1 and dose $5x10^8$iu as DNA=2.5. Nonmem VI and Berkeley-Madonna software programmes were used to build the semi-mechanistic models. Non parametric bootstrap was used to calculate the 95% confidence intervals of the parameter estimated. Graphics were plot using R programme.

Model A

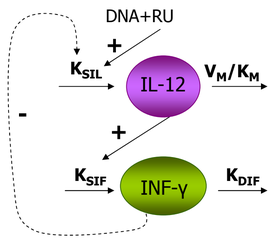
$$\frac{d(IL12)}{dt} = K_{SIL} \times \left(1 + \frac{DNA \times RU \times SLRU}{1 + REG}\right) - K_{DIL} \times IL12$$
$$t = 0 \rightarrow IL12 = IL\_0$$

$$\frac{dIFN\gamma}{dt} = K_{SIF} \times \left(\frac{IL12}{IL12 + A50}\right) \times RU - K_{DIF} \times IFN\gamma$$
$$t = 0 \rightarrow IFN\gamma = 0$$

$$\frac{dREG}{dt} = K_{REG} \times IFN\gamma - K_{REG} \times REG$$
$$t = 0 \rightarrow REG = 0$$



Model B



$$\frac{d(IL12)}{dt} = K_{SIL} \times \left(1 + \frac{DNA \times RU \times SLRU}{1 + IFN\gamma}\right) - \frac{V_M \times IL12}{K_M + IL12}$$
$$t = 0 \rightarrow IL12 = IL\_0$$

$$\frac{dIFN\gamma}{dt} = K_{SIF} \times \left(\frac{IL12}{IL12 + A50}\right) \times RU - K_{DIF} \times IFN\gamma$$
$$t = 0 \rightarrow IFN\gamma = 0$$

Model C

$$\frac{dIL12}{dt} = K_{SIL} \times \left(1 + \frac{DNA \times RU \times SLRU}{1 + IFN\gamma}\right) - K_{ON} \times IL12 \times R$$
$$t = 0 \rightarrow IL12 = IL\_0$$

$$\frac{dR}{dt} = K_{SYN} - K_{ON} \times IL12 \times R - K_{DEG} \times R$$
$$t = 0 \rightarrow R = R_{MAX}\_0 - RIL12\_0$$

$$\frac{dRIL12}{dt} = K_{ON} * IL12 * R - K_{INT} * RIL12$$
$$t = 0 \rightarrow RIL12 = RIL12\_0$$

$$\frac{dIFN\gamma}{dt} = K_{SIF} \times (RIL12 - RIL12\_0) - K_{DIF} \times IFN\gamma$$
$$t = 0 \rightarrow IFN\gamma = 0$$

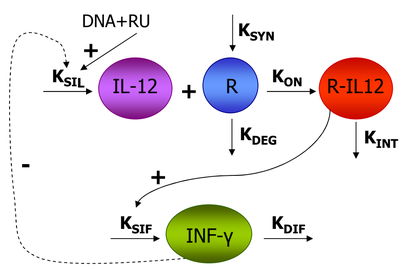


Figure 2. Scheme and mathematical equations for the three models developed. K_{SIL}: IL-12 zero order synthesis rate constant; K_{DIL}: IL-12 degradation constant, SLRU: Slope induced by the administration of RU; K_{REG}: modulator rate constant; V_M: maximal elimination rate constant; K_M: amount of IL-12 at which V_M is half-maximal; K_{SIF}: IFNγ synthesis; A50: half maximal inhibitory amount of IL-12; K_{DIF}: IFNγ degradation rate constant; IL_0: basal IL-12 levels; K_{SYN}: zero order receptor synthesis rate constant; K_{DEG}: zero order receptor degradation rate constant; R_{MAX}_0: basal amount of total receptor; RIL12_0: basal amount of bound receptor; K_{ON}: receptor binding second order rate constant; K_{INT}: internalization rate constant.

III. Analysis of the model

Berkeley-Madonna was used to explore the receptor dynamic at different doses and the behaviour of model C when IFNγ receptor levels were compromised (knock-out mice). Degradation of IFNγ rate constant was modified by a factor (I) ranging from 1 (normal receptor levels) to 0 (no receptor levels) and simulations were plot against real data obtained from knock-out mice for the IFNγ receptor.

Results

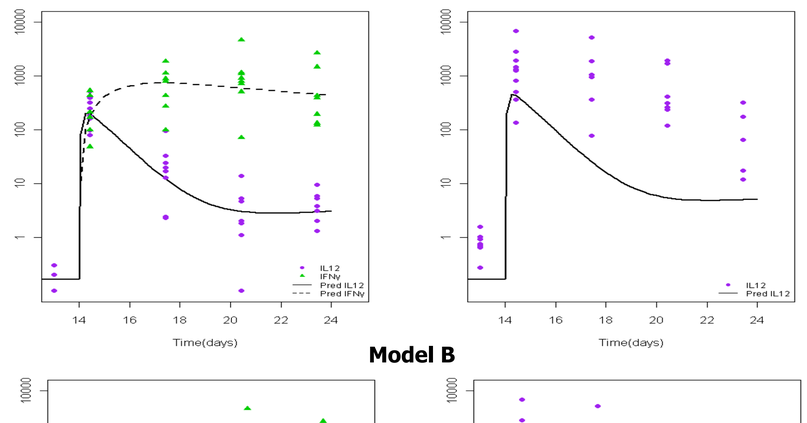
Model A was able to describe the kinetics of IL-12 and IFNγ at the lower dose; however the profiles predicted for the higher dose underestimated the observations (Figure 3, Model A). A non linear pharmacokinetic was proposed, and a Michaelis-Menten degradation of the IL-12 was implemented (Figure 3, Model B). When applying model B, the increased levels of IFNγ could be describe using a direct effect and no delay compartment (Modulator) was needed. Looking for a more physiological model, the concept of targeted-mediated drug disposition was explored and incorporated using Berkeley-Madonna software (Figure 3, Model C); IL-12 bounds to free receptor in the surface membrane of the cells and induces the synthesis of IFNγ. Subsequently, the complex is internalized and eliminated.

Table 1. Pharmacokinetic parameters of the three models proposed

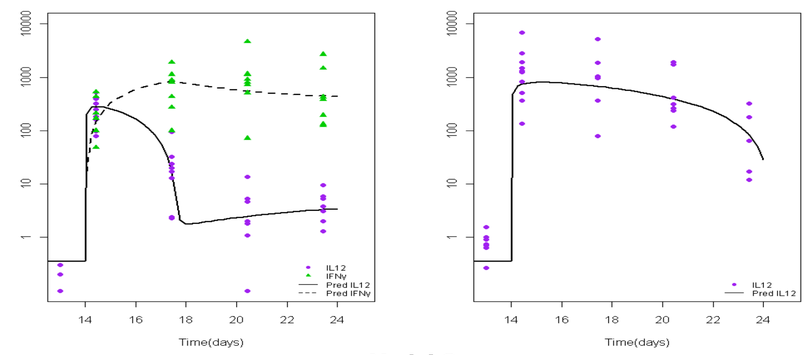
Parameter (units)	Model A [a]	Model B [a]	Model C [a]
K_{SIL} (ng/day)	0.183 (0.174-0.192)	9.24 (3.03-46.2)	7.35 [b]
IL_0 (ng)	0.162 (0.154-0.17)	0.331 (0.189-0.513)	0.332 (0.188-0.536)
SLRU (pg/iu)	9670 (9420-9920)	2530 (612-8100)	2790 (1240-6026)
K_{SIF} (pg/(ng*day))	651 (386-916)	396 (236-668)	1.46 (0.454-3.96)
K_{DIF} (day^{-1})	0.136 (0.0845-0.188)	0.216 (0.132-0.345)	0.549 (0.288-2.06)
K_{REG} (day^{-1})	0.476 (0.468-0.484)	-	-
A50 (ng)	74.3 (73.4-75.2)	12.7 (1.37-42.3)	-
K_M (ng)	-	5.55 (1.15-18.2)	-
V_M (ng/day)	-	164 [b]	-
K_{ON} (ng/day)	-	-	0.0603 (0.021-0.23)
RIL_0 (ng)	-	-	24.1 (8.12-147.2)
R_{MAX}_0 (ng)	-	-	391 (154-1046)
K_{DEG} (day^{-1})	-	-	0.457 (0.248-1.12)
K_{INT} (day^{-1})	-	-	0.305 [b]
K_{SYN} (ng/day)	-	-	175 [b]

[a] Parameter value (95% confidence interval)
[b] Parameter calculated: $V_M = K_{SIL} \times (K_M + IL\_0)/IL\_0$; $K_{SIL} = K_{ON} \times IL\_0 \times (R_{MAX}\_0 - RIL12\_0)$, $K_{INT} = K_{SIL}/RIL\_0$; $K_{SYN} = K_{SIL} + K_{DEG} \times (RMAX\_0 - RIL12\_0)$

Model A



Model B



Model C

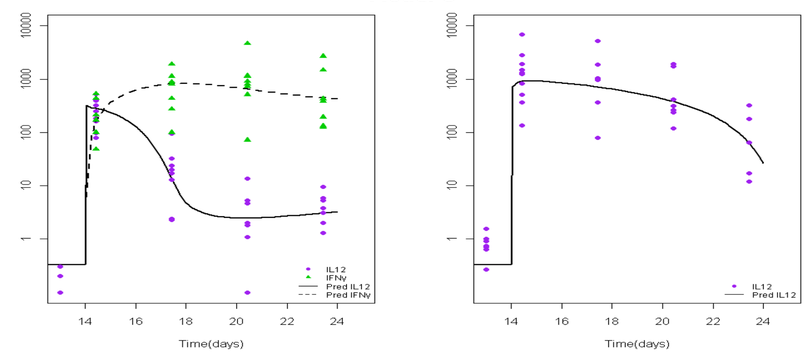


Figure 3. Observations of IFNγ (green triangles) and IL-12 (purple circles) along with the predictions of IFNγ (dashed lines) and IL-12 (solid lines) for each one of the three models. Left panels represent the lower dose (DNA=1). Right panels represent the observation and prediction of IL-12 for each one of the three models for the higher dose (DNA=2.5).

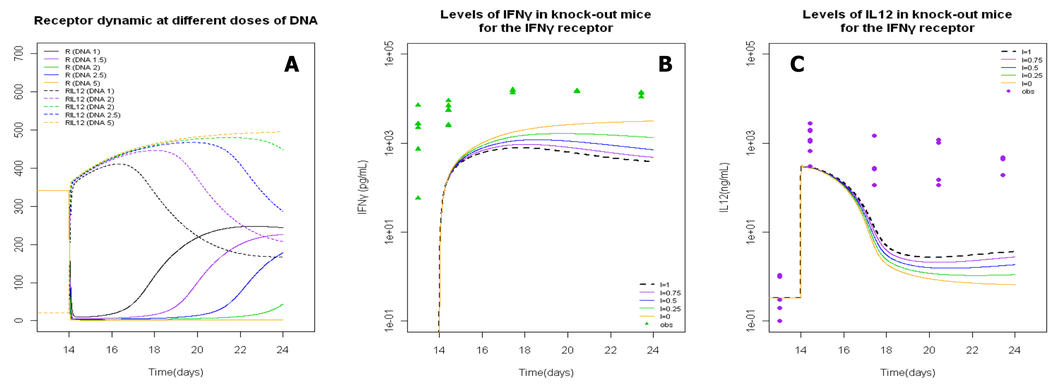


Figure 4. A: Free receptor (solid lines) and bound receptor (dashed lines) dynamic. B: Observations of IFNγ (green triangles) plotted against IFNγ simulated profiles of model C when K_{DIF} is modified by a factor (I) ranging from 0 to 1. C: Observations of IL-12 (purple points) plotted against IL12 simulated profiles of model C when K_{DIF} is modified by a factor (I) ranging from 0 to 1.

Model C was not able to describe the increased levels of IFNγ and IL-12 observed when IFNγ degradation was decreased (Figure4, B & C). A new model incorporating the targeted-mediated drug disposition for the IFNγ was then explored using Berkeley-Madonna. This new model was able to describe both the increased levels of IL-12 and of IFNγ observed in knock out mice (Figure 5). Therefore, a new model for wild-type and knock-out mice with different basal levels of IFNγ and where targeted-mediated drug disposition is considered for both molecules (IL-12 and IFNγ) has been proposed (Figure 6).

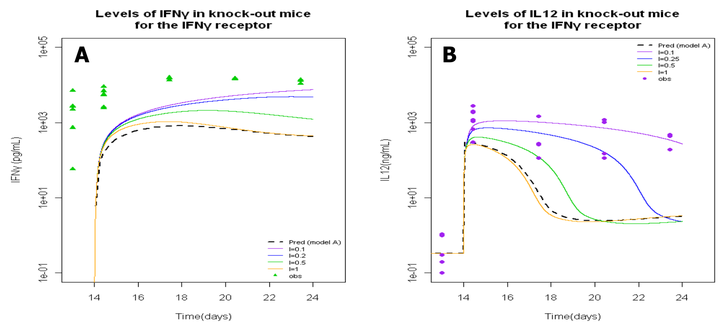


Figure 5. A: Observations of IFNγ (green triangles) plotted against IFNγ simulated profiles of model D when K_{DIF} is modified by a factor (I) ranging from 0 to 1, dashed lines represent model C predictions when I=1. B: Observations of IL-12 (purple points) plotted against IL-12 simulated profiles of model C when K_{DIF} is modified by a factor (I) ranging from 0 to 1, dashed lines represent model C predictions when I=1.

$$\frac{dIL12}{dt} = K_{SIL} \times \left(1 + \frac{DNA \times RU \times SLRU}{1 + RIFN\gamma}\right) - K_{ON} \times IL12 \times R$$
$$t = 0 \rightarrow IL12 = IL\_0$$

$$\frac{dR}{dt} = K_{SYN} - K_{ON} \times IL12 \times R - K_{DEG} \times R$$
$$t = 0 \rightarrow R = R_{MAX}\_0 - RIL12\_0$$

$$\frac{dRIL12}{dt} = K_{ON} * IL12 * R - K_{INT} * RIL12$$
$$t = 0 \rightarrow RIL12 = RIL12\_0$$

$$\frac{dIFN\gamma}{dt} = K_{SIF} \times RIL12 - K'_{ON} \times I \times IFN\gamma \times R'$$
$$t = 0 \rightarrow IFN\gamma = IFN\gamma\_0$$

$$\frac{dR'}{dt} = K'_{SYN} - K'_{ON} \times IFN\gamma \times R' - K'_{DEG} \times R'$$
$$t = 0 \rightarrow R = R'_{MAX}\_0 - RIFN\gamma\_0$$

$$\frac{dRIFN\gamma}{dt} = K'_{ON} \times I \times IFN\gamma \times R' - K_{DIF} \times RIFN\gamma$$
$$t = 0 \rightarrow RIFN\gamma = RIFN\gamma\_0$$

Figure 6. Mathematical equations of model D. K_{SIL}: IL-12 zero order synthesis constant; SLRU: Slope induced by the administration of RU; K_{SYN}: IL-12 receptor synthesis zero order rate constant; K_{DEG}: IL-12 receptor zero order degradation rate constant; R: free receptors of IL-12; RIL12_0: basal amount of bound receptor; K_{ON}: receptor-IL-12 binding second order rate constant; K_{INT}: RIL12 internalization rate constant; K_{SIF}: IFNγ synthesis; K'_{ON}: receptor-IFNγ binding second rate constant; IFNγ_0: basal IFNγ levels; K'_{SYN}: IFNγ receptor zero order synthesis rate constant; K'_{DEG}: IFNγ receptor zero order degradation rate constant; R': free receptors of IFNγ; RIFNγ_0: basal amount of bound receptor; K_{DIF}: RIFNγ degradation rate constant

Conclusions

Berkeley-Madonna was used to develop two non-linear pharmacokinetic models and to explore the concept of targeted-mediated drug disposition. Both models will be further improved by using data of knock-out mice for the IFNγ receptor.

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