European Journal of Pharmaceutical Sciences 37 (2009) 418–426

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Semi-mechanistic pharmacodynamic modelling of gene expression and silencing processes

Pedro Berraondo[a], Gloria González-Aseguinolaza[a], Iñaki F. Trocóniz[b,*]

[a] Division of Hepatology and Gene Therapy, Center for Investigation in Applied Medicine (CIMA), Avda. Pío XII 55, 31008 Pamplona, Spain
[b] Department of Pharmacy and Pharmaceutical Technology, School of Pharmacy, University of Navarra, Irunlarrea 1, Pamplona 31080, Spain

ARTICLE INFO

Article history:
Received 13 October 2008
Received in revised form 7 February 2009
Accepted 25 March 2009
Available online 5 April 2009

Keywords:
Semi-mechanistic modelling
Gene therapy
Gene expression and silencing
Interferon α
NONMEM

ABSTRACT

Background: Suppressed expression of transgene is a major obstacle in gene therapy. Understanding of the mechanisms involved in expression and silencing of exogenous genes is required to overcome gene therapy hurdles.
Purpose: To develop a semi-mechanistic model describing the effects of transgenes over the activity of an expression cassette.
Methods: Twelve Balb/c mice received 40 μg of plasmid DNA. Animals were assigned to one of the following treatments: (I) 20 μg of the plasmid expressing luciferase (pEF-Luc) and 20 μg of "empty" plasmid; (II) pEF-Luc (20 μg) and 20 μg of plasmid expressing murine interferon alpha (IFNα); and (III) pEF-Luc (20 μg), and 20 μg of plasmid expressing β-galactosidase (pCMVβ). The expression of luciferase over time, quantified by a noninvasive method, was used as a measured of pEF-Luc activity and modelled using NONMEM.
Results: The selected model suggests the co-existence of two forms of active DNA differing in their transcription efficiencies. The core model was expanded to describe reversible and irreversible silencing processes, induced by the coexpression of IFNα or β-galactosidase, respectively.
Conclusion: Coupling noninvasive *in vivo* imaging and mathematical modelling allows quantitative description of gene transfer, providing a tool to select the best regulatory elements to construct a therapeutic expression cassette.

1. Introduction

The pharmacokinetic and pharmacodynamic attributes of gene therapy products are not well understood. Several gene therapy protocols have failed in part due to the absence of data regarding the behaviour of an expression cassette in the presence of the therapeutic transgene. For example, the use of long-term expression vectors to express certain therapeutic genes, such as cytokines, has been hampered due to the elimination of transgene expression (Berraondo et al., 2005; Reboredo et al., 2008). This elimination can be mediated by inactivation or inhibition of the promoter that control the expression (Al-Dosari et al., 2006). Alternatively, the abrogation of transgene expression may be due to physical elimination of plasmid or cells bearing exogenous DNA (Aubert et al., 2002; Mingozzi et al., 2007). Thus, the success of gene therapy strategies requires the correct selection of regulatory elements to control gene expression.

Mathematical modelling may provide a theoretical framework where different interpretations of experimental data can be tested. Although the modelling approach has been used in the past to analyze gene therapy derived data (Varga et al., 2001, 2005; Banks et al., 2003; Kamiya et al., 2003), greater efforts in this area would be desirable, since for example and to our knowledge such quantitative approach has not yet applied to *in vivo* data and to reversible/irreversible perturbation of gene expression.

IFNα is a key element in the defense against viral and other pathologies (Biron, 2001). IFNα signalling is initiated by binding to the cell surface receptors, IFNAR1 and IFNAR2, and is known to be mediated by the Janus kinase signal transducer and activation of transcription (Jak-Stat) pathway and the subsequent induction of hundreds of genes with antiviral and antiproliferative properties (Der et al., 1998; Stark et al., 1998). IFNα has been approved for use in a number of indications including chronic viral infection and cancer. However, response rate to recombinant IFNα is far from satisfactory and the therapy is not devoid of unwanted side effects. To overcome those limitations, new ways of IFNα delivery, such as the use of gene therapy (Berraondo et al., 2005) are currently under development with the aim of increasing the efficacy of the

* Corresponding author. Tel.: +34 616 788 025; fax: +34 948 42 56 49.
E-mail address: itroconiz@unav.es (I.F. Trocóniz).

0928-0987/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejps.2009.03.013

IFNα therapy. However, as has been recently described, interferon stimulated genes (ISG) inhibit promoter activity as well as cell protein synthesis (Qin et al., 1997; Der et al., 1998). Therefore, IFNα produced by a gene therapy vector may inhibit its own production, leading to a negative feed-back. This effect can be overcome by choosing the right promoter or modifying it in order to make a given promoter non-sensitive to the inhibitory actions of ISGs (Aubert et al., 2002; von Marschall et al., 2003; Mian et al., 2005).

In this work, naked DNA was administered by hydrodynamic injection, directing the plasmid to liver parenchymal cells (Liu et al., 1999; Zhang et al., 1999). The objective of the current study is to describe quantitatively using semi-mechanistic models the gene transcription efficiency of a plasmid expressing luciferase under the control of the selected promoter with or without a plasmid expressing interferon alpha (IFNα), or a plasmid expressing the highly immunogenic protein β-galactosidase to stimulate antigen specific cytotoxic immune response leading to the clearance of the transduced hepatocytes. This mathematical approach allows to explore the performance of models based on different physiological mechanisms and discrimination between system and non-system model parameters.

2. Methods

2.1. Plasmid construction, production and purification

The construction of the plasmid, pEF-Luc, encoding firefly luciferase under the control of the human Elongation Factor 1α (hEF1α) promoter has been described previously (Berraondo et al., 2005). Murine IFNα-1 gene was amplified by PCR using the following primers (forward: 5′-GAAGCTTTGGCAACACTCACC-3′; reverse: 5′-CTCTACACTTTGGCTCAGGACTC-3′). The PCR product was purified and cloned into pcDNA3.1 following manufacturers' instructions (Invitrogen, Carlsbad, CA). The IFNα gene was sequenced and cloned into pGTC1100 (kindly provided by Dr. C. Qian, CIMA, Spain), under the control of the hEF1α promoter. The plasmid pCMVβ (Clontech, Palo Alto, CA) expresses β-galactosidase driven by CMV promoter.

Plasmids were amplified in the DH5α strain of *E. coli* was extracted and purified by a QIAGEN Endofree Plasmid Giga kit (QIAGEN GmbH, Hilden, Germany). The purity was checked by 1% agarose gel electrophoresis followed by ethidium bromide staining. Plasmid concentration was measured by UV absorption at 260 nm.

2.2. Plasmid administration

Twelve female Balb/c mice aging 8 weeks and weighing between 18 and 20 g received a total amount of 40 μg of plasmid DNA by hydrodynamic injection, consisting in the administration of 1.8–2.0 ml of the DNA solution via tail vein in five seconds (Der et al., 1998; Biron, 2001). DNA injections were performed in the morning and the animals had food and water *ad libitum*. Animals were randomly assigned to one of the following treatment groups: group I, where the mice received 20 μg of the plasmid expressing luciferase (pEF-Luc) and 20 μg of "empty" plasmid that does not contains any gene (pGTC1100); group II, where two plasmids were administered, pEF-Luc (20 μg) and other expressing murine IFNα (pEF-mIFNα) (20 μg); and group III, where the mice were injected with two plasmids pEF-Luc (20 μg), and the other expressing β-galactosidase (pCMVβ) (20 μg). All animal procedures were conducted under institutional that comply with national laws and policies.

2.3. Bioluminescence measurement

The response variable measured was the levels of luciferase activity (LUC) as an indicator of the expression of luciferase gene from the plasmid pEF-Luc ($mRNA_{LUC}$). LUC was measured 6 h after injection and every 2–3 days thereafter for a period of 30–33 days.

Mice were anaesthetized using ketamine/xilacine and 100 μl of D-luciferine (Xenogen, Alameda, CA) at a concentration of 30 mg/ml diluted in 150 mM NaCl solution were injected by intraperitoneal route. The animals were placed in the imaging chamber of the Xenogen IVIS system (Xenogen Corp., Alameda, CA), which includes a cooled CCD camera. A gray-scale photograph of the animals was acquired, followed by a bioluminescent acquisition. Regions of interest (ROIs) were drawn over the positions of greatest signal intensity on the animal, as well as over regions of 'no' signal which were used as background readings. Light intensity was quantified using photons/second/cm^2/sr [luciferase activity (LUC)]. The gray-scale photograph and data images from all studies were superimposed using LivingImage (Xenogen Corp., Alameda, CA).

Supplementary material (Figure a) shows as an example the *in vivo* imaging of luciferase expression after hydrodynamic injection. A representative mouse of each group is shown at day 7 (A) and at day 26 (B).

2.4. Data analysis

The population approach using the software NONMEM version VI with the first order conditional estimation method with INTERACTION was used for the analyses (Beal et al., 2006). Data were logarithmically transformed for the analysis. Inter-animal variability (IAV) was modelled exponentially and residual variability was described with an additive error model. Model parameters are presented as the estimates together with their coefficients of variation [CV(%)]. The degree of IAV was also expressed as CV(%).

Selection between models was based on the precision of parameter estimates, goodness-of-fit plots, and the value of the Akaike's Information Criteria (AIC) computed as $-2LL + 2 \times N_p$, where $-2LL$ corresponds to the minimum value of the objective function [$-2\log$(likelihood); $-2LL$] provided by NONMEM, and N_p is the number of the parameters in the model (Akaike, 1974). The model with the lowest value of AIC, given that precision of model parameters and data description was adequate, was selected.

The final selected was explored using the visual predictive check (Karlsson and Holford, 2008). One thousand luciferase activities versus time profiles were simulated for each group maintaining the same design conditions. Simulated values were obtained at each day of the period of study (33 days). Then, for each observation time the 0.05, 0.5, and 0.95 percentiles of the simulated values were computed and plotted together with the corresponding raw data. The agreement between model-based simulations and observed values was judged visually.

Model development was driven by the data which were presented in Fig. 1. Our starting point was the theoretical models proposed by Kamiya et al. (2003), for gene therapy where the possibility of having different degrees of active DNA was presented. In the following only the model finally selected is described in detail. Fig. 2 provides a schematic representation of the selected model, built using models and concepts commonly applied in the field of the pharmacokinetic/pharmacodynamic modelling such as the models for (i) delay compartments (Sheiner et al., 1979), (ii) indirect responses (Dayneka et al., 1993), (iii) competitive reversible interactions (Porchet et al., 1988), and (iv) irreversible effects (Jusko, 1971).