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EXPERT REVIEW

Gene Therapy: A Pharmacokinetic/Pharmacodynamic Modelling Overview

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ABSTRACT Since gene therapy started over 20 years ago, more than one-thousand clinical trials have been carried out. Nonviral vectors present interesting properties for their clinical application, but their efficiency *in vivo* is relatively low, and further improvements in these vectors are needed. Elucidating how nonviral vectors behave at the intracellular level is enlightening for vector improvement and optimization. Model-based approach is a powerful tool to understand and describe the different processes that gene transfer systems should overcome inside the body. Model-based approach allows for proposing and predicting the effect of parameter changes on the overall gene therapy response, as well as the known application of the pharmacokinetic/pharmacodynamic modelling in conventional therapies. The objective of this paper is to critically review the works in which the time-course of naked or formulated DNA have been quantitatively studied or modelled.

KEY WORDS computational modelling · gene therapy · pharmacokinetic/pharmacodynamic modelling

INTRODUCTION

During the last two decades, pharmacokinetic/pharmacodynamic (PK/PD) models for both efficacy and safety have been developed in almost all therapeutic areas (1–3), showing a clear benefit in the drug development process (4).

There are areas, however, where PK/PD modelling is still an exception. One of those areas is gene therapy (5). Gene therapy can be defined as the transfer of genetic material (DNA or RNA) to somatic cells in order to obtain a therapeutic effect, by either (i) correcting genetic defects, (ii) over-expressing proteins that are therapeutically useful, or (iii) inhibiting the production of harmful proteins (6).

Since 1989, when the first clinical trial was approved for advanced melanoma (7), over 1500 clinical trials have been carried out or approved (8). The major percentage (89.1%) of these trials focuses on cancer therapies, cardiovascular diseases, infectious diseases (acquired immune deficiency syndrome (AIDS)) and inherited monogenic diseases (cystic fibrosis). However, although there are two gene therapy drugs commercialized for cancer disease in China, no human gene therapy product has been approved by the Food and Drug Administration (FDA) or European Medicines Agency (EMEA) to date (9).

The concept of *drug* is continuously evolving, from pills to intelligent drug delivery systems and from natural products to biotechnology (proteins, DNA, RNA or even cells). Yet, it can be expected that if models have been proved to be beneficial in understanding and predicting the effects of "traditional" drugs, the same would be the case for modern therapies such gene therapy (10).

(Semi-)mechanistic PK/PD models have to take into consideration the following: (*i*) formulation characteristics, (*ii*) biopharmaceutic aspects, (*iii*) pharmacokinetic and pharmacodynamic properties, and (*iv*) system behaviour. All those aspects can be represented by processes that can be quantified by estimating specific parameters through a model-building process using experimental and/or simulated data.

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Ledley *et al.* (11) proposed a series of processes, which in the case of being quantitatively characterized by a set of parameters, would allow for describing and understanding the kinetics of the effects elicited by the gene therapy system. Those processes were (*i*) distribution and biological fate of the DNA expression vector, (*ii*) efficiency of DNA uptake into cells, (*iii*) dynamics of intracellular trafficking, (*iv*) degradation of the DNA vector in the cell, (*v*) transcription rate from DNA to mRNA, (*vi*) mRNA stability, (*vii*) translation rate of the mRNA to create the gene product, (*viii*) intracellular compartmentalization or secretion of the gene product, and (*ix*) pharmacokinetics of the gene product in the body.

The same authors introduced the terms *intrinsic* and *apparent kinetics*. *Intrinsic kinetics* refers to processes *i* to *viii* mentioned above. The *apparent kinetics* of the gene therapy product would be reflected by the time course of the synthesised therapeutic protein and represented by process *ix* above.

Developing mechanistic models requires a great amount of experimental and computational resources. The rationale for such investment resides in the possibility to explore by computer simulations the impact of specific modification in the vectors and in the time course of the therapeutic response once the model has been developed and the key intrinsic kinetics mostly affecting the apparent kinetics identified.

The objective of this manuscript is to review and discuss the most relevant works where the time course of the genetic material administered and its mediated response has been (semi-)mechanistically modelled with model parameters estimated from the experimental data or obtained from the available literature. In the following sections of the manuscript, models describing the intrinsic kinetics processes mentioned above are presented and discussed.

SYSTEMIC AND ORGAN PHARMACOKINETICS

Naked Plasmid DNA (pDNA)

Administration of naked DNA into the body is the simplest means of gene therapy. Wolff *et al.* (12) were the first in obtaining gene expression levels after intramuscular injection of naked DNA. However, a conventional intravenous injection of pDNA results in low or undetectable transgene expression in major organs (13). The reason for this low efficiency can be found in the physicochemical and biological properties of the DNA. DNA is a big molecule with a molecular weight over 2000 kDa and strong anionic charge and is easily degraded by the existing DNases in the blood. Therefore, its permanence and distribution in the body are limited (14,15). Understanding the *in vivo* fate of DNA itself is a prerequisite to develop safe and efficient gene delivery systems.

Houk *et al.* studied the kinetics of naked pDNA in rat plasma (*ex vivo*) (16), and also *in vivo* after intravenous administration of supercoiled DNA (17), developing an integrated model capable to describe simultaneously the time course of the three functional forms of pDNA: supercoiled (SC), open circle (OC) and linear (L).

The model developed by the authors linked the SC, OC and L topoforms using ordinary differential equations represented by the following scheme: SC → OC → L →. In the case of the *ex vivo* experiments, the kinetics of the system were modelled using first-order processes with estimates of half-lives of 1.2, 21, and 11 min, respectively, resembling the activity of nucleases residing in the plasma. Based on their *in vivo* results, an additional irreversible loss of the SC from the central compartment, representing tissue uptake, was incorporated into the model, as well as a Michaelis-Menten (MM) process to account for the formation of the L topoform from OC. The *in vivo* estimates of the elimination half-lives in plasma for the SC, OC, and L topoforms were 0.15, 11 (at concentrations of OC lower that the MM constant), and 21 min, respectively, values not far from those obtained in the *ex vivo* study.

Plasmid DNA kinetics have been further investigated both qualitatively and quantitatively using different approaches (13,18,19). From the different studies it can be concluded that naked pDNA has a short half-life after intravenous administration and is rapidly cleared from the plasma by both enzymatic degradation and organ uptake, preferably by liver (20,21). Nevertheless, the amount of DNA greatly diminished from tissues within hours, explaining, along with the plasma nucleases activity, the low, if any, levels of transgene expression observed after simple intravenous administration (14).

Different attempts to increase the transduction efficiency of naked DNA have been made (22), such as the use of electroporation techniques (23,24), intravascular delivery or hydrodynamic injection (25,26). Those attempts try to overcome the systemic elimination mechanisms and increase tissue bioavailability of the genetic material. Less invasive alternatives as the development of viral and nonviral delivery systems that protect pDNA from systemic elimination and facilitate tissue uptake represent currently one of the major research focus in gene therapy.

Formulated pDNA

Tissue distribution of the gene therapy system is essential, since transgene expression only occurs in those cells transfected with the genetic material. *In vivo*, tissue distribution is determined by the physicochemical and biological properties of the vector employed. Therefore, formulation, along with the route of administration, is crucial to achieve the therapeutic objectives. Fig. 1

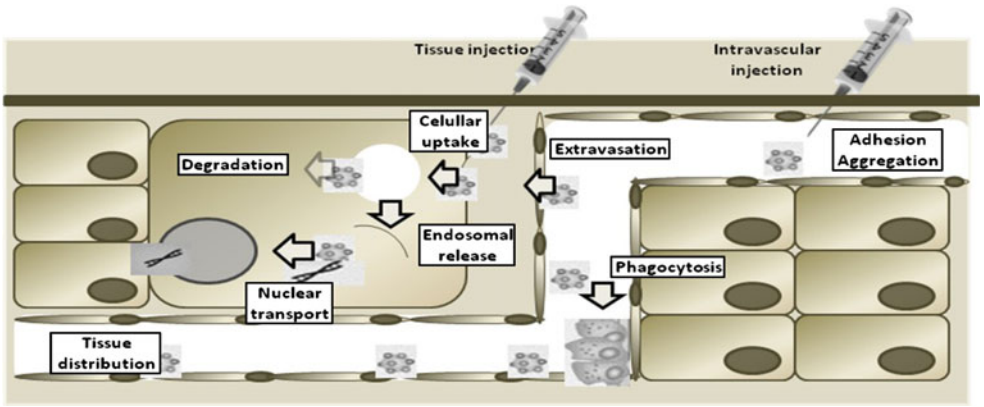

Fig. 1 Barriers in gene therapy after *in vivo* administration [adapted from ref (13)]

summarizes the main body barriers found after *in vivo* gene administration.

Viral vectors, predominantly retroviruses and adenoviruses, are the most used vectors in clinical trials, due to greater efficiency when compared with nonviral delivery systems (i.e., cationic complexes). Currently, approximately two-thirds of the clinical trials performed to date have used viral vectors (27).

Relative inefficiency of transfection for nonviral vectors compared to viral counterparts remains the largest barrier to synthetic vector development and application. However, these vectors present some advantages over the viral ones that make their investigation and improvement particularly valuable for clinicians and scientists (28). Nonviral vectors are safer, less immunogenic, more cost-effective and easier to produce; they also have fewer practical limitations in the size of the transgene, which are very promising features for *in vivo* applications (29). The key to improving the clinical outcomes of transfection with nonviral techniques includes optimizing the parameters involved in gene delivery and expression while maintaining the advantages of the synthetic vectors.

Cationic lipid/ DNA complexes have been proved to be rapidly cleared from the bloodstream after intravenous (iv) injection in general terms (28,30,31), accumulating primarily in lung and liver, and to a less extent in spleen. Nevertheless, there is redistribution between these two first organs, with an initial accumulation in lung, followed by a gradual increase in liver (19,32). This observation has been explained by a first-pass effect: in the presence of serum components, lipoplexes could form aggregates being passively targeted to pulmonary microvasculature, the first capillary bed encountered after iv injection. The hepatic redistribution would be due to complex dissociation and small complex carried away by blood flow from the lung (32,33). The use of different co-helpers lipids, lipid: DNA charge ratio and size have been proved to influence tissue distribution (13).

Tissue distribution of polyplexes is more easily controlled since cationic polymers interact less with blood components. Thus, targeted delivery can be achieved by controlling the physicochemical and biological properties of the complex (14). Jeong *et al.* (34) showed the influence of molecular weight (MW), structure and Nitrogen/ Phosphate (N/P) ratio in the biodistribution and tissue expression kinetics after iv administration of pDNA complexed with polyethylenimine PEI. These authors detected a prolonged retention of pDNA in liver when high MW or branched PEI were used, while linear PEI exhibited higher levels in the lung at the same molecular weight.

Although an important number of studies have quantified the temporal course of different nonviral systems (10,19,30,34), modelling efforts to describe the systemic pharmacokinetics of formulated pDNA are very sparse. Yu *et al.* (32) used a two-compartment model to describe the time course of naked and Terplex DNA (lipopolyplex). The same model was used recently by Zhou *et al.* (35) in their comparative study between naked DNA and three cationic polymer complexes.

Table I summarizes some of the studies in which pharmacokinetics of pDNA (naked or complexed) have been studied after iv administration. Differences in the estimates of half-life ($t_{1/2}$) reported between studies can be attributed mostly to differences in the method of measurement and to species variation along with different techniques employed for the quantification of DNA.

As in the case of the systemic pharmacokinetics, and despite that in several articles levels of the genetic material administered are measured and reported in different organs at different times after administration, not many efforts have been made to relate the time course in plasma with the corresponding in tissues. This is the case, for example, of the works published by Lew *et al.* (30) and Li *et al.* (36). That kind of exercise is worth being taken into consideration if, for example, levels of genetic material have to be predicted in humans based on data obtained in different animal species, as it is done in the case of standard therapeutics. Moreover, different mechanisms of tissue uptake and elimination can be compared by fitting different physiologic-based models to plasma and tissue data.

Table I Summary of the Studies In Which pDNA Time Course has been Quantified After Intravenous Administration

Ref	DNA description	Species/ model	Quantification Method	pDNA Dose	Formulation	Parameters	$t_{1/2}$ (min)
(18)	Chloramphenicol acetyltransferase gene fused to SV 40 promoter	Mice	[α-^{32}P] labelling	1 mg/kg	Naked DNA	AUC, Cl, Tissue uptake	~10 *in vitro* NE *in vivo*
(31)	Chloramphenicol acetyltransferase gene	Mice	[α-^{32}P] labelling	0.1 mg/kg	DOTAP/DOPE/pDNA DDAB/DOPE/pDNA	AUC, Cl, Tissue uptake	–
(30)	pDNA encoding firefly luciferase under the control of CMV promoter	Mice	Southern blot analysis	50 μg	DMRIE/DOPE/pDNA	Data were no model	SC: ND OC and L: <5
(28)	Plasmid containing luciferase under the control of RSV promoter	Mice	Agarose gel analysis	75 μg	pDNA pDNA/DOGS/ DOPE	Data were no model	SC: ND SC: NE, OC: 10-20
(13)	pDNA codifying for the luciferase under the control of the CMV promoter	Mice	[^{33}P]labelling	45 μg	Linear naked pDNA DDAB/DOPE/ pDNA	Data were no model	6.6–11.5 4.3–8
(19)	pDNA encoding chloramphenicol acetyl transferase	Mice	[^{33}P]labelling	3 mg/kg	Naked pDNA DOTIM: chol pDNA	Data were no model	–
(16)	pGL3 (commercial plasmid)	Rat plasma	Fluorescence	12 μg	Naked pDNA	Degradation rates, Cl, AUC	SC:1.2, OC: 21 L: 11
(17)	SC naked pDNA	Rats	Fluorescence	2500 μg 500 μg	Naked pDNA DOTAP:chol:pDNA	Non-compartmental analysis (AUC, CL, V,MRT Cmax, Tmax)	SC: 0.15, OC: 11.09, L: 21.48 SC: 0.69
(32)	pDNA	Rats	[^{32}P] labelling	10 pmol	Naked pDNA stearyl-PLL/low density lipoprotein(LDL)	Two-compartmental model (AUC, Vd, Cl, MRT)	117.6 370.8
(34)	Plasmid encoding for murine interleukin-2 under the control of the CMV promoter	Mice	PCR	50 μg	Naked DNA 25Kd PEI/pDNA	AUC, MRT	1.76 7.57
(35)	gWiz^{TM} high expression luciferasa	Mice	Real time-PCR	1 μg /kg	Naked pDNA rPLL/ pDNA rSDN/pDNA RGD-rSDN/pDNA	Two-compartment and non-compartment model (AUC, CL, Vd, MRT…)	3.47 3.12 16.84 15.87

SV40: Simian Virus 40; CMV: Cytomegalovirus; DDAB: dimethyldioctadecylammonium bromide; DOTAP: N-(2,3-bis(oleyloxy)propyl)-N,N, trimethylmmonium chloride; DOPE: dioleoylphosphatidylethanolamine; DOTIM: 1-[2-[9-(Z)-octadecenoyloxyl-ethyl]]-2-[8-(Z)-heptadecenyl]-3-[hydroxyethylenyl]imidazolynium chloride; DOGS: optimized formulation of lipospermine; chol:cholesterol; PEI: polyetylenimine; PLL: reversible polylisine; LDL: low density lipoprotein; rPLL: reversible PLL; rSDN: reversible stabilized nanoparticles; RGD-rSDN arginina-glycine-aspartic acid targeted polyplex; PCR: polimeras chain reaction; RT-PCR: real time- PCR; AUC: area under the curve; Vd: distribution volume; CL: clearance; NE: not estimated; ND: not quantifiable

In this context of tissue pharmacokinetics, the models developed by Nomura *et al.* (37) and recently by Mok *et al.* (38) represent an interesting and valuable approach to understand the fate of the gene therapy systems in a more controlled environment compared to systemic (iv or oral) administration. In both cases, administration of the genetic material was intratumoral.

Nomura *et al.* (37) established a two-compartment model based on ordinary differential equations to study the intratumoral behavior of naked pDNA and its cationic liposome complexed in an ovarian tumor model system, detecting an enhanced tissue retention when liposomes were used and identifying the transfer rate of poorly perfused tumor region to the well-perfused one as the main factor for this retention. However, complex formation did not improve gene expression because of its poor dissemination in this tumor.

The diffusion, internalization, and degradation processes of the Herpes Simplex oncovirus in human soft tissue sarcoma were modeled in the work of Mok *et al.* (38). The model identified the rapid binding and internalization and the low diffusion as the main causes of the low distribution of the virus in the tumor. Those results led the authors to

suggest technological modifications as alteration of the viral envelope, or alteration of the interstitial space to overcome the main limitations.

INTRACELLULAR PHARMACOKINETICS

Once the administrated pDNA has arrived at its target, efficiency can be further enhanced by modifying intracellular disposition (39). The use of fusogenic lipids (e.g. DOPE: dioleoylphosphatidylethanolamine) (40) or molecules with buffering capacities (e.g. PEI) (41) (to disrupt the endosomal membrane and increase pDNA released to the cytoplasm), the incorporation of nuclear localization signal (NLS) (42) to facilitate nuclear delivery, or the selection of appropriate promoters have been proved to increase nonviral vectors efficiency. Understanding the intracellular dynamics of genetic material is, therefore, instructive for vector improvement.

Mathematical models to characterize and quantitatively describe the intracellular processes of nonviral or viral gene expression systems can be developed to identify the main events controlling the desired transgene expression. Furthermore, these intracellular kinetic models would allow us to propose and predict the effect of variations of multiple parameters at once, representing a useful strategy for optimizing the intracellular trafficking of gene delivery (43). Table II collects examples of intracellular mathematical models developed.

Intracellular uptake and delivery of the plasmid DNA is a multi-step process across several cellular regions: endocitosis is the predominant mechanism of uptake for both viral and nonviral vectors. Once in the cell, subcellular trafficking leads the transport of internalized complexes through early and late endosomes to lysosomal degradation of the delivered therapeutic. To overcome this destruction, the complexes must escape from the lysosome, and different strategies have been developed to do so, as has been previously commented. Once in the cytoplasm, the complex can dissociate, and the free DNA can enter into the nucleus for gene transcription to take place, but also the whole complex can penetrate through nuclear pore complexes and dissociate in the nucleus. Meanwhile, nucleases present in the cytoplasm serve to rapidly degrade unprotected plasmids; thus, it may be necessary to ensure plasmid protection through control of vector-plasmid dissociation kinetic.

Ledley *et al.* (11) were the first to propose a three-compartmental model to describe the kinetics of the intracellular DNA (milieu, endosome and cytoplasm). Their model showed that certain processes, such as degradation of endosomal and intracellular DNA or degradation of mRNA, could be described with conventional first-order kinetics. Applying the model developed, the authors studied

Table II Intracellular Mathematical Models Developed for Nonviral Vectors

Ref.	Data	Model	Expression system	Rate-limiting process
(11)	Literature data	Six-compartment model	Computer simulation	DNA, RNA or product stability
(44)	Literature and experimental data	First-order mass action kinetic model	Plasmid encoding for GFP modified under the controlled CMV complexed with Lipofectamine	Nuclear binding import and nuclear pore association
(45)	Experimental data	Three-compartimental model based on concepts of mass action kinetics	pDNA codifying for GFP formulated with cationic lipid to create a lipoplex	Nuclear translocation of intact plasmid
(48)	Literature and experimental data	First-order mass action kinetic model, based on the model previously developed in 2001	Plasmid encoding the β-galactosidase gene under CMV promoter naked or complexed with an adenoviral vector, PEI (2 KDa, 25 KDa or dodecylated 2 KDa) or Lipofectamine	Endosomal escape for naked DNA and both 2 KDa PEI, nuclear import for adenovirus and 25 KDaPEI, and cell uptake for Lipofectamine
(49)	Experimental data	Multicompartment mathematical model as consecutive first-order mass action events	Plasmid encoding the interleukin 12 gene under CMV promoter complexed with PEI (1.8 KDa, 25 KDa or cholesterol 1.8 KDa)	Endosomal escape, nuclear entry/ membrane binding, and endosomal uptake, respectively

the effects of different intrinsic kinetic parameters in the expression level and demonstrated that it was not only a function of the promoter strength and the efficiency of gene transfer into the cell (29) (transfection), but also the intrinsic stability of the DNA, RNA and protein express.

A more complex model was developed and validated by Varga *et al.* (44) using data from previous works as well as their own experimental studies to describe the intracellular processes, differentiating between the cytoplasm and the nucleus compartments.

The major drawback to Varga's model was the use of data obtained under different experimental conditions and cell lines. Trying to overcome this inconvenience, Banks *et al.* (45) developed a three-compartmental model to estimate the rate constants of DNA transport (active and passive) across cellular and nuclear membranes using two *in vitro* cell systems: HeLa (human epitheliod cells) and CV1 (monkey fibroblast cells). The translocation rate constants for the intact plasmid were different for each cell system; however, the cytoplasm-nucleus transport was slower than the extracellular-cytoplasm one in both cell lines. Those results led the authors to suggest nuclear delivery as the main limiting step in nonviral gene expression.

One of the main limitations when developing an intracellular pharmacokinetic model is the data availability. The little information available is mainly due to the lack of an adequate assay system for quantifying the disposition of pDNA in each organelle. General procedures imply a separation of the different subcellular fractions followed by a PCR and/or Southern blotting quantification. Nevertheless, there is always some uncertainty in the recovery and the possibility of leakage between endosomal and cytoplasmic fraction. Akita *et al.* (46) have developed a new strategy to simply and reliably quantify the intracellular dynamics of rhodamine-labelled pDNA in the cytosol, endosome, and nucleus simultaneously for nonviral gene delivery systems using images captured by confocal laser scanning microscopy. This group applied the new technique of intracellular disposition described previously to compare two nonviral vectors developed in their laboratory with the commercially available transfection reagent Lipofectamine Plus (Invitrogen, CA, USA) and a viral vector (adenovirus) in order to optimize them by determining the rate-limiting process from a kinetic point of view (47).

Despite the limitations previously described, authors such as Varga (48), mentioned above, or Zhou (49) have been able to develop and apply different mathematical models to describe the intracellular kinetics of nonviral vectors (Fig. 2a). Similarly, some models have been developed for viral systems, although are not discussed in this review (50,51). The development of these intracellular models allows for quantitatively elucidating the rate-limiting steps for a variety of vector/cell systems and to predict the effect of parameter changes on gene expression. Table II collects some of the models in which the intracellular steps have been studied along with the conclusions achieved. A summary of the parameters obtained in the different nonviral intracellular models described is displayed in Table III. Two types of parameters can be drawn from the table: (*i*) parameters, as cell internalization or cytoplasmic degradation, that remain similar through the different plasmid/cell system experimental conditions (despite the vector system studied), and (ii) parameters, as is the case of endosomal escape or degradation, which are determined by the physicochemical properties of the vector employed. As can be seen in Table III, chemical modifications of these vector properties, such as the incorporation of dodecyl carbon chain (PEI 2C12) or cholesterol molecules proposed by the authors, improved the intracellular performance of the gene delivery system. Estimates by Ledley *et al.* (11) have not been included in the table due to the dissimilar processes characterized.

PHARMACODYNAMICS

So far, models to describe systemic, organ/tissue, and intracellular events and exposure have been reviewed and discussed. However, the link between exposure and effect (synthesis of the therapeutic protein) is still lacking.

The disposition of the DNA inside the nucleus is determined by its origin; the DNA can integrate into host's genome (main characteristic of retroviruses) or maintain an extrachromosomal location. Upon integration, vector genes appear to be expressed for a long period; however, it may induce carcinogenesis (52). On the other hand, extrachromosomal DNA is progressively reduced in the number of copies by cellular division and loss by degradation generating a transient expression (39). Regardless of the disposition, DNA has to be transcribed to mRNA, which will then be exported to the cytoplasm and traduced into its encoding protein. At this level, gene expression is going to be regulated by different factors, such as the disposition, the plasmid stability in the nucleus or the DNA expression cassette used (promoter/enhancer, poly (A), etc.) (53). Kamiya *et al.* (43) introduced in 2003 the new concept of "controlled intranuclear disposition," essential to understanding nuclear DNA fate and achieving desirable gene expression.

As in the case of works reviewed in previous sections, models dealing with the time course of the *in vivo* response are not frequent. Nevertheless, results presented from a non-model perspective have shown the non-linear characteristics between gene exposure and effect.

Different groups have studied quantitatively the correlation between pDNA dose or nuclear DNA and transgene expression. Tachibana *et al.* (54) evaluated the relationship between plasmid delivery to the nucleus using liposome

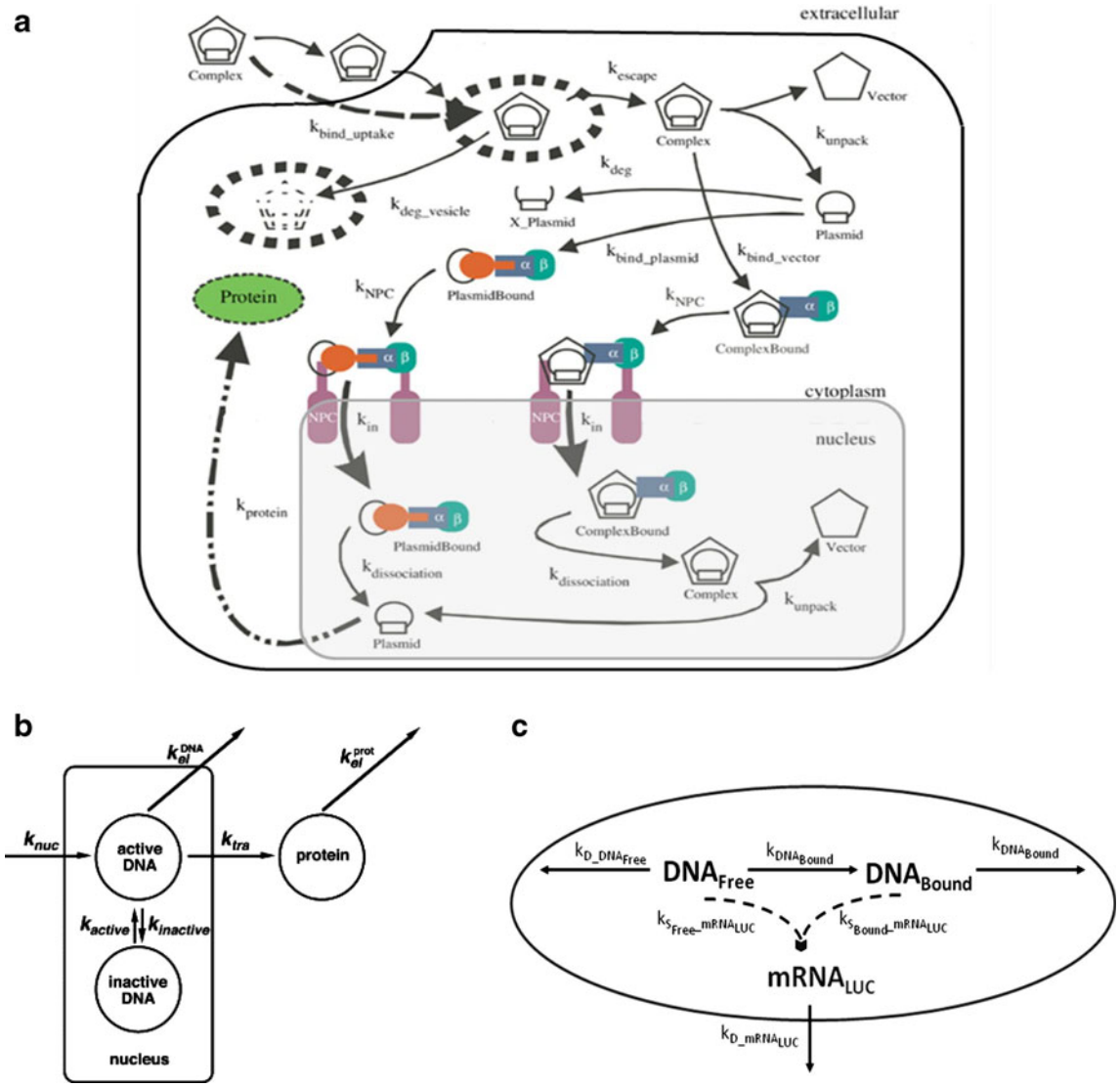

Fig. 2 **a** Intracellular pharmacokinetics following endocitosis of the delivery system [adapted from ref (48)]. **b** Intranuclear PK/PD model: after entering the nucleus (k_{nuc}), the active form of DNA can be transcripted and translated into a protein (Ktra) and inhibited by reversible conversion to inactive DNA ($k_{inactive}/k_{active}$) or elimination (k_{el}) [adapted from ref (56)]. **c** Intranuclear PK/PD model where both DNA forms can be translated to mRNA, though with different rates, and eliminated from the cell [adapted from ref (57)]

complexes, observing a dose-dependent increase in nuclear uptake along with a saturation of gene expression against intranuclear plasmids.

Moriguchi *et al.* (55) studied this phenomenon and found also a remarkable positive non-linear relationship between dose and transfection activities in nonviral gene delivery systems *in vitro*. This non-linearity was attributed to PD processes (nuclear stability, transcriptional efficiency, etc.), since insignificant differences in the pharmacokinetic processes were detected at different doses and conditions employed (dumb DNA and empty liposomes). Further studies of the same group observed that the number of DNA copies in the nucleus of one cell appeared to be fundamental in determining the gene expression levels, and suggested that DNA can work synergistically inside the nucleus, explaining this non-linear relationship (56).

Similar conclusions were achieved by Hama *et al.* (47) when studying adenovirus (Ad) and Lipofectamine (LFN). The authors observed that, although intracellular PK was similar between these two systems, to achieve a comparable transgene expression, LFN required three more orders of intranuclear DNA copies than Ad due to the higher transcription efficiency of Ad (8100 times higher). These results lead Hama *et al.* (47) to hypothesize that DNA condensation was an important factor regulating transcriptional processes.

Semi-mechanistic models describing the time course of the gene therapy have been proposed recently by Yamada

Table III Estimates of Intracellular Pharmacokinetic Models Developed for Nonviral Gene Therapy

	Varga (2001)	Banks (2003)		Varga (2005)						Zhou (2009)		
		Lipoplex		Ad5	PEI 25	PEI 2	PEI2C12	LFN	ND	PEI 25	PEI 1.8	WSLP
k_{MP}	.	$^{a}3.1\times10^{-2}$	$^{a}3.8\times10^{-3}$	$^{b}6.0\times10^{-3}$	$^{b}6.0\times10^{-3}$	$^{b}5.0\times10^{-3}$	$^{b}7.0\times10^{-3}$	$^{b}7.0\times10^{-3}$	$^{b}2.0\times10^{-3}$	1.0×10^{-5}	1.7×10^{-5}	5.8×10^{-3}
k_{PE}	.									8.4×10^{-3}	2.8×10^{-3}	3.8×10^{-3}
k_{escape}	1.0×10^{-2}	.	.	1.6×10^{-2}	1.0×10^{-2}	5.0×10^{-5}	6.0×10^{4}	.	1.0×10^{-5}	1.6	1.7×10^{-6}	9.7×10^{-3}
k_{unpack}	1.0×10^{9}	.	.	1.0×10^{-2}	5.0×10^{-2}	5.0×10^{-1}	8.0×10^{2}	.	.			
$k_{bind\_plasmid}$	2.0×10^{-3}	.	.	2.0×10^{-3}	2.0×10^{-3}	2.0×10^{-3}	2.0×10^{-3}	2.0×10^{-3}	2.0×10^{-3}			
k_{bind_vector}	.	.	.	1.0×10^{-1}	4.0×10^{-2}	1.0×10^{-5}	2.0×10^{2}	.	.	$^{c}1.5\times10^{-3}$	$^{c}5.9\times10^{-2}$	$^{c}3.6\times10^{-2}$
k_{NPC}	1.0×10^{3}	$^{a}6.1\times10^{-1}$	$^{a}8.7\times10^{-2}$	1.0×10^{3}	1.0×10^{3}	1.0×10^{3}	1.0×10^{3}	1.0×10^{3}	1.0×10^{3}			
k_{in}	3.0×10^{-3}			3.0×10^{-3}	3.0×10^{-3}	3.0×10^{-3}	3.0×10^{-3}	3.0×10^{-3}	3.0×10^{-3}			
$k_{dissociation}$	1.0×10^{3}	.	.	1.0×10^{3}	1.0×10^{3}	1.0×10^{3}	1.0×10^{3}	1.0×10^{3}	1.0×10^{3}			
k_{drug_veside}	.	.	.	2.0×10^{-2}	1.5×10^{-1}	1.0×10^{-2}	2.0×10^{2}	2.0×10^{-2}	2.0×10^{2}	9.9×10^{-3}	1.3×10^{-1}	2.6×10^{-6}
k_{deg}	5.0×10^{-3}	.	.	5.0×10^{-3}	5.0×10^{-3}	5.0×10^{-3}	5.0×10^{-3}	5.0×10^{-3}	5.0×10^{-3}	7.7×10^{-2}	7.3×10^{-2}	1.9×10^{-2}
$k_{protein}$	1.0×10^{-2}	.	.	.	.	.	.	.	.	.	.	.

Ad5: Adenovirus; PEI 25: polyethylenimine 25 KDa; PEI 2: polyethylenimine 2 KDa; PEI 2C12 dodecylated polyethylenimine 25 KDa; LFN:lipofectamine; ND: naked DNA; PEI 1.8: polyethylenimine 1.8 KDa; WSLP: polyethylenimine 25 KDa conjugation of cholesterol chloroformate; k_{MP}: membrane binding rate constant; k_{PE}: endosomal uptake rate constant; k_{escape}: endosomal escape rate constant; k_{unpack}: DNA unpack rate constant; $k_{bind\_plasmid}$: nuclear import protein binding to plasmid rate constant; k_{bind_vector}: nuclear import protein binding to vector rate constant; k_{NPC}: nuclear pore complex rate constant; k_{in}: translocation into the nuclear compartment rate constant; $k_{dissociation}$: dissociation of nuclear import protein from plasmid or vector; $k_{drug\_vesicle}$: plasmid degradation (endosomal) rate constant; k_{deg}: degradation of free plasmid in the cytosol; $k_{protein}$: gene expression rate constant. [a] Constant rate accounting for active and passive transport across the corresponding membrane and considered as the sum of k_{MP} and k_{PE}. [b] Constant rate calculated k_{bind_uptake} considered as the sum of k_{MP}; k_{PE}; [c] Constant rate calculated KCN considered as the sum of k_{unpack}, $k_{bind\_plasmid}$, k_{bind_vector}, k_{NPC}; K_{in}; $K_{dissociation}$. Units are expressed in min^{-1}

et al. (57) and Berraondo *et al.* (58). In their work, Yamada *et al.* (57) studied *in vitro* the luciferase kinetic production after transfection with cationic lipids by measuring luciferase activity at different time points. Based on their results, and on the general model presented by Kamiya *et al.* (43), the authors proposed a preliminary model (Fig. 2b) in which the amount of intracellular protein might be controlled by the processes represented by: the nuclear entry (k_{nuc}), activation and inactivation of transcription (k_{active} and $k_{inactive}$), transcription and translation efficiency (k_{tra}) and elimination of both exogenous DNA (k_{el}^{DNA}) and cellular protein (k_{el}^{prot}). However, the corresponding model parameters were not computationally calculated.

Berraondo *et al.* (58) developed a semi-mechanistic model to describe *in vivo* the kinetics of luciferase expression after administration of naked DNA by hydrodynamic injection (Fig. 2c) under different system perturbation (reversible and irreversible gene expression inhibition). Taking as well the model of Kamiya *et al.* (39) as a starting-point, intranuclear pharmacokinetic processes were inferred from the luciferase activity measures: in the nucleus, free DNA could be degraded ($k_{D\_DNAFree}$) or stabilized by transforming to bound DNA ($k_{DNABound}$); therefore, two DNA forms were proposed to coexist in the nucleus, although with different transcriptional rates ($k_{SFree\_mRNALUC}$ and $k_{SBound\_mRNALUC}$), and finally the mRNA was eliminated from the cell ($k_{D\_mRNALUC}$). It is noteworthy that the estimated parameters of DNA free degradation and protein synthesis of their model ($k_{D\_DNAFree}$: $1.6\times10^{-3}\text{min}^{-1}$; $k_{SFree\_mRNALUC}$ + $k_{SBound\_mRNALUC}$: 4.8×10^{-2} min^{-1}) presented values of the same orders as the estimates reported by the models developed by Varga *et al.*(44) (Table III).

Recently, Ruponen *et al.* (59) estimated the elimination half-life of intracellular pDNA for a series of non-viral vectors. In their manuscript, the time course of luciferase activity was also measured but not modeled as a function of the intracellular DNA. The authors conclude that there was a poor correlation between intracellular DNA release/elimination and transgene expression. Applying models as those developed by Berraondo *et al.* (58), which are in their time profiles similar to the Ruponen *et al.* (59), might be an alternative worth considering to reconcile pDNA fate with gene expression, besides having the possibility to test through different models and to explore different hypothesis about the mechanism of gene expression.

SUMMARY

Published works featuring distribution of naked/complex DNA, plasma, tissue and intracellular pharmacokinetic as well as pharmacodynamic of different gene therapy vectors have been reviewed and discussed. Although it has been shown how PK and PD can help in the optimization and rational development of nonviral gene therapies, to our knowledge, non-integrated PK/PD model has not been developed yet.

From our perspective, there are two fundamental aspects that have to be taken into consideration when integrating the modelling approach with the goal of optimizing and understanding gene therapy response. The first accounts for intracellular kinetics of the therapeutic DNA, and the second deals with the dynamics of the gene expression elicited. The study of the intracellular disposition events will help during the analysis of the gene expression data to discriminate between those events that are related with DNA disposition in the cell from those related with other events, like silencing mechanisms or triggering immune response, that mainly depends on the gene expression itself. Cell disposition of DNA can be studied *in vitro* using cell cultures (Banks *et al.* (45)) and the results can be coupled to the model in development for the *in vitro/in vivo* gene expression data measured through imaging techniques allowing continuous monitoring over time (Berraondo *et al.* (58)). System perturbation is a key issue to understanding how and why a particular biological system reacts. Administration of different DNA loads, together with the use of different promoters, are parts of the ideal experimental design.

It is anticipated that the knowledge gathered from a modelling-oriented experiment will help to understand the time course of the *in vivo* effects (including inter-subject variability) as it has already been proved in the case of more standard therapeutic approaches.

Given the complexity of the techniques employed and the knowledge required to accomplish the *in vitro/in vivo* experiments, along with the expertise needed to develop a mathematical model, it is necessary to establish multidisciplinary collaboration between experimental and modelling areas to be able to integrate the concepts of PK/PD modelling in gene therapy.

These types of collaborative approaches are worthy not only for a better understanding of the biological processes regulating gene therapy responses, but also for optimizing preclinical and clinical phases in the development of new therapeutic agents.

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