

From in vitro-in vivo relationship (IVIVR) towards in vitro-in vivo extrapolation (IVIVE): A case study of pulmonary delivery systems

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ABSTRACT

Based on the results from the literature, a direct relationship between dissolution profiles and in vivo markers of low molecular weight heparin (LMWH) concentration was discovered by application of the RIVIVR tool. Previously, LMWH-loaded microspheres were administered intratracheally to rats and anti-factor Xa activity was measured as a surrogate of LMWH actual concentration in blood. A non-compendial dissolution method was employed to produce in vitro dissolution profiles. Four microsphere formulations were taken into consideration, and RIVIVR was employed to produce direct relationships between dissolution profiles and anti-Xa factor activity of heparin. Four experiments were performed, and each time one formulation was excluded for external validation. Internal and external validation errors were computed as prediction errors (PEs) (%). In the case of all four formulations, all of PEs were below 10%, both in internal and external validation procedures. The results indicate good quality of the models and flexibility of the RIVIVR tool, whereas IVIVR (in vitro-in vivo relationship) is built without intravenous administration data. Moreover, as anti-Xa factor activity is no direct measurement of heparin concentration, the results confirm usability of RIVIVR for nonclassical pharmacokinetic data for the in vivo part of IVIVR.

KEYWORDS: In vitro-in vivo correlation (IVIVC); in vitro-in vivo relationship (IVIVR); pulmonary delivery system; dissolution

BACKGROUND

Pulmonary drug delivery is an effective way of drug administration during the treatment of the respiratory diseases as well as systematic diseases. When a drug is acting as a systematic agent, one can treat such approach as a noninvasive 'needle-free' delivery system. Pulmonary delivery is suitable for a variety of small substances and is especially useful when drugs with high first-pass metabolism are considered. An environment independent of diet-driven modifications with low enzymatic activity, and free of hepatic or intestinal first-pass effect, assures reproducible absorption kinetics. Additionally, low lung clearance allows for applying sustained-release formulations with prolonged release of a drug (1, 2).

A reproducible and reliable method of modeling the drug bioperformance is needed if pulmonary delivery of the systemic agent is to be considered. Moreover, in this environment, non-linear relationships should be expected if pharmacokinetic (PK) data are being predicted. RIVIVR, a tool described previously, was applied to model the data obtained from literature with low molecular weight

heparin (LMWH) considered as a model systemic agent (3, 4). RIVIVR, as an empirical modeling tool, can handle such data without going into mechanistic details of the process if enough data are provided from in vivo and in vitro studies.

Generally, IVIVR (in vitro-in vivo relationship) is an effort to relate in vitro dissolution data to in vivo PK data, and IVIVE (in vitro-in vivo extrapolation) is a broader term that aims to predict phenomena on full living organisms based on experimental results or observations. In this case, an actual pharmacodynamic (PD) parameter was modeled because anti-Xa activity, which describes the anticoagulant properties of heparin, was measured. Thus, in our approach, a direct relationship between dissolution profiles and in vivo markers of LMWH concentration was investigated, as a surrogate of actual LMWH concentration in blood.

METHODOLOGY

Data

Data were obtained from studying the feasibility of large porous particles as long-acting carriers for pulmonary delivery of LMWH. Poly(DL-lactide-co-glycolide) (PLGA)

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microspheres were loaded with LMWH of 61/mg anti-factor Xa activity. Particles were obtained with an evaporation method using dichloromethane as an evaporating solvent. Obtained microspheres were washed with distilled water and lyophilized (freeze-dried) to obtain free-flowing powder (4).

Dissolution study

A non-compendial dissolution method was employed to produce in vitro dissolution profiles. The amount of 50 mg of freeze-dried microspheres was suspended in 10 mL phosphate-buffered saline with the addition of 0.1% polysorbate 80. Magnetic stirring of 200 rpm was employed. Subsequently, samples were taken and were centrifuged. Supernatant was removed and assayed for heparin content with a colorimetric method.

In vivo study

Male Sprague–Dawley rats (Charles River Laboratories, Charlotte, NC, USA) were used to measure in vivo phenomena. Measurements were taken indirectly, through plasma anti-factor Xa activity.

The animal plasma was added to a known amount of excess factor Xa. If heparin is present in the plasma, it binds to antithrombin and forms a complex with factor Xa. The amount of residual factor Xa is inversely proportional to the amount of heparin. The residual factor Xa is detected by adding a substrate that mimics the natural substrate of factor Xa. This is cleaved by the residual factor Xa, releasing a colored compound (chromophore) that can be detected by a spectrophotometer, resulting in the anti-factor Xa activity profile.

The study microspheres were administered intratracheally (50 U/kg) by using a specially designed dry-powder insufflator (Penn Century, Philadelphia, PA, USA) for aerosol inhalation in small animals. Plasma obtained from untreated rats was used as a negative control to account for the effect of rat endogenous anti-factor Xa that otherwise could give a false positive increase in anti-factor Xa activity.

As stated by the authors of the original study, correlation of the in vitro release with the release of API in the lungs can be challenging since the medium used for in vitro tests differed significantly from the actual lung fluid (5). However, with the empirical approach presented here, if the model is validated, it can be assumed that predictability can be achieved on the satisfactory level.

Four formulations were considered, named PM-2, PM-SP-2, PM-SA-2, and PM-PEI-2 (4). The mean diameters of the particles were 6.77, 6.65, 9.85, and 11.01 μm ,

respectively. Each one of them consisted of 50 mg of LMWH encapsulated in 250 mg of PLGA microspheres, which differed in terms of the internal aqueous phase (IAP) to organic phase (OP) to external aqueous phase (EAP) ratio used to prepare the microspheres, which was 0.25:5:25 for PM-2 and 0.5:5:25 for other three formulations, respectively. PM-SP-2, PM-SA-2, and PM-PEI-2 contained core-modifying agents in the IAP: sorbitan monostearate to stearylamine to polyethyleneimine, respectively.

Modeling procedure

Similarly to the case of IVIV correlations, an assumption was made that a direct relationship between in vitro and in vivo data can be established (5).

RIVIVR is a new tool for a direct, convolution-based, correlation of dissolution profiles with their PK counterparts. It is based on the optimization approach where optimized parameters are points of numerically developed intravenous (IV) time-concentration curve (in silico IV profile) used for convolution of the PK profile representing oral administration of the particular formulation. Once optimized on two or three available formulations, the in silico IV profile is used for convolution-based prediction of a new formulation PK profile based on the new dissolution profile, thus accounting for the external validation.

The algorithm of RIVIVR execution is represented by following pseudo-code:

- 1: Gather in vitro and in vivo data (at least 2 formulations with different release rates)
- 2: Step = 1
- 3: **IF** step = 1, **THEN**
 - initialize artificial IVIV profile (impulse)
- ELSE**
 - update artificial IVIV profile from p. 5
- 4: Step = step +1
- 5: Modify artificial IVIV profile
- 6: Convolve artificial IVIV profile with in vitro profile
- 7: Compare convolved PK profile with its corresponding observed counterpart
- 8: Compute prediction error upon comparison
- 9: **IF** prediction error > stop criterion, **THEN**
 - go back to p. 3
- ELSE**
 - exit.

Four experiments were performed, and each time one formulation was excluded for external validation. Internal and external validation errors were computed as prediction errors (PEs) (%).

RESULTS AND DISCUSSION

In the case of all studied formulations, PEs were below 10%, both in internal and external validation procedure. This is a confirmation of good quality of the models. Table 1 shows external PEs.

Table 1. Prediction Errors (PE) for the External Validation Procedure

Formulation	R ²	PE_Cmax (%)	PE_AUC (%)
PM-2	0.795	8.22	0.14
PM-SP-2	0.841	3.92	2.07
PM-SA-2	0.941	5.37	1.84
PM-PEI-2	0.995	1.52	0.95

Profiles given as an input differed between each other, which guarantees good generalization abilities of the prediction model. Predicted profiles of Xa activity are presented in Figure 1.

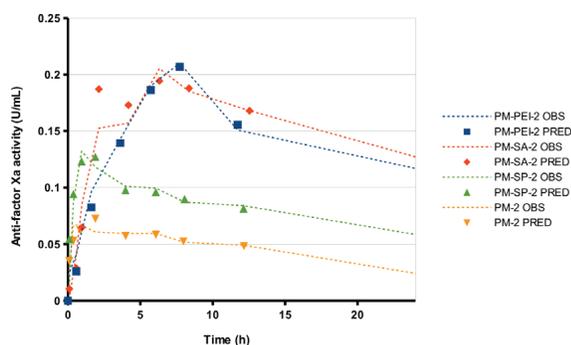


Figure 1. Predicted (PRED) anti-factor Xa activity profiles and observed (OBS) values.

Figure 2 shows the predicted versus observed plots with R-squared for each prediction validation and the overall, which was 0.948, again demonstrating good quality of predictions.

On Figure 3, the summary of steps mentioned above are depicted. In each experiment, only three formulations were used to calculate and optimize artificial impulse IV profile, upon which the Xa activity was predicted. Presented calculated impulse profiles (Figure 4) have no physical meaning, being only a scaling factor for the numerical convolution procedure. As RIVIVR is purely empirical tool, loss of mechanistic understanding of the “impulse” curve is a price for good predictability of the model described above.

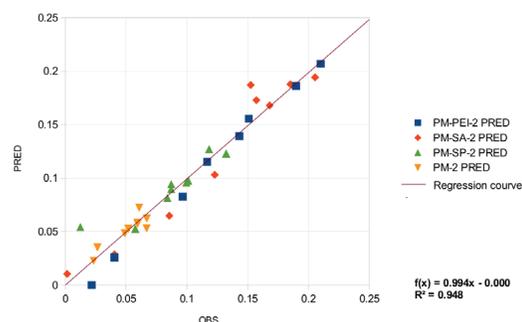


Figure 2. Predicted (PRED) versus observed (OBS) scatter-plot for all formulations. Solid line presents linear model and R² of all formulations.

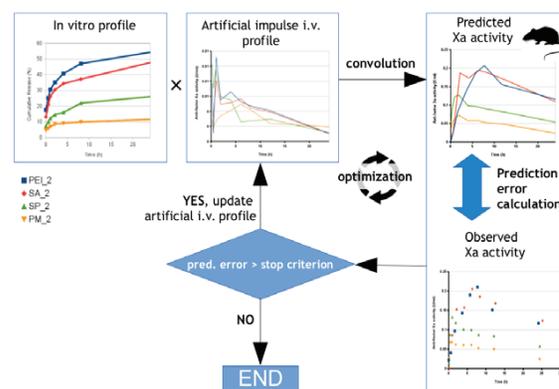


Figure 3. Optimization procedure explained on the given dataset.

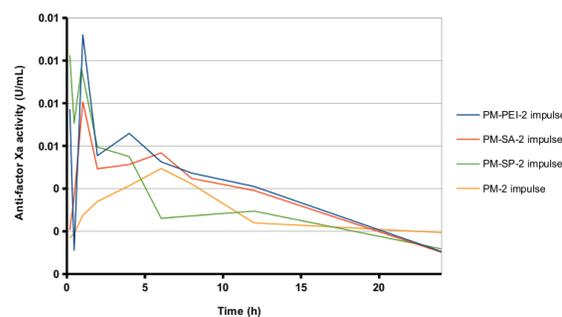


Figure 4. Generated impulse profiles.

CONCLUSIONS

We have demonstrated the usefulness of the RIVIVR tool in the modeling of the complex relationship between in vitro and in vivo data. Results indicate good quality of the models and flexibility of the RIVIVR tool, whereas IVIVR is built without intravenous administration data. Moreover, as anti-Xa factor activity is no direct measurement of heparin concentration, the above results confirm usability of RIVIVR for nonclassical PK data for the in vivo part of IVIVR.

The results of the current study correspond well to the recent advancements in model-based drug development (6, 7). In general, the in vitro–in vivo extrapolation relates to either PK or PD effects scaling from in vitro-measured ADME (absorption, distribution, metabolism and excretion) parameters or compound activity data for PK and PD, respectively, to the in vivo situation (8, 9). The novel approach presented here offers new possibilities in the situation when limited data are available.

It is noteworthy that not only PK but also pharmacodynamic effects were predicted in this case, solely based on microspheres dissolution data. Therefore, a simple in vitro/PD model was established.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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