Novel *in vitro* PK (non-animal PK) Assay for Excipient/Vehicle Formulation Studies

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ABSTRACT

A new assay has been developed for fast *in vitro* screening of the effect of excipients (e.g., 2-hydroxypropyl-β-cyclodextrin (HP-β-CD), polyethylene glycol 400 (PEG400), etc.) on absorption of sparingly soluble compounds.

INTRODUCTION

A research project done in collaboration between *p*ION INC and F. Hoffmann-La Roche studying the effect of excipients on equilibrium solubility[1] and permeability[2] (PAMPA) of mostly sparingly soluble compounds concluded that the excipients' influence on both properties together must be considered when trying to predict a particular excipient's effect on absorption.

While separately measuring solubility and PAMPA with and without excipients has great informative value[3], it is quite labor intensive as well as time and compound consuming because it requires several PAMPA and solubility assays to be performed per compound to assess the effect of various excipients on absorption.

A new *in vitro* assay was developed (*patent pending*[4]), which, similarly to pharmaco-kinetic (PK) studies, ranks excipients based on their effect on absorption without individually measuring the permeability and solubility of compounds.

MATERIALS AND METHODS

The appearance rate of a compound in the receiving compartment of a 2-chamber artificial phospholipid barrier permeation system containing excipient in the donor compartment was compared to the corresponding rate in an excipient-free system. The system and reagents consisted of a 96-well Double-Sink™ PAMPA Sandwich with pre-loaded stirrers, a PRISMA™ donor buffer adjusted to pH 5.0, 6.2, and 7.4 with and without excipients, GIT-0 artificial membrane lipid, and Acceptor Sink Buffer (ASB-7.4) in the receiving compartment (*p*ION INC). Fig. 1 schematically illustrates the setup of an *in vitro* PK assay:

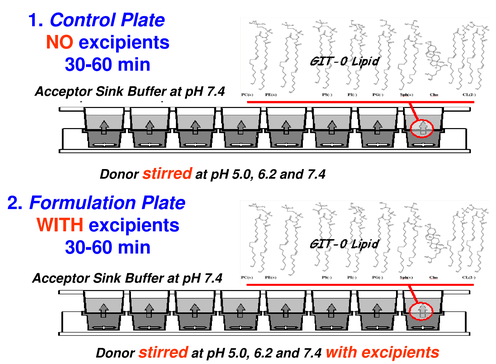

Fig. 1. *In vitro* PK setup: Two "sandwiches" where the only difference between them is the presence of excipient in the donor compartments of one of them (patent pending[3]).

After introducing compound in the donor compartment, only the UV signal of the acceptor is collected after 30 minutes of incubation time. Calibrated for *in vivo* conditions, individual-well stirring was provided by the Gut-Box™ (pION INC.)

Concentrations of excipients mimicked those in the gastro-intestinal tract rather than in formulations.

The effect of a particular excipient on the compound was assessed using a ratio (the Flux Ratio, FR) of the concentrations in two acceptor compartments where their donor compartments were with and without the excipient.

RESULTS AND DISCUSSION

Because the method does not require measuring the concentration in the donor compartments, sparingly soluble compounds can be effectively studied for excipient compatibility. Whether the presence of excipient in the solution improves or impedes the absorption properties of compounds, or does not change them at all, can be learned in the matter of hours by recognizing the four possible outcome cases in terms of the Flux Ratio (FR):

Case 1: FR ~ 1 or log(FR) ~ 0
Case 2: FR < 1 or log(FR) < 0
Case 3: FR > 1 or log(FR) > 0
Case 4: FR is undetermined

For example, Fig. 2a illustrates Case 2 (FR < 1), where the excipient causes a decrease in permeability is possibly the dominant effect.

The most exciting case is illustrated in Fig. 2b where FR is raised 7-fold. This outcome means that the excipient has a positive overall effect on absorption, most likely due to a substantial improvement in the solubility properties of the compound.

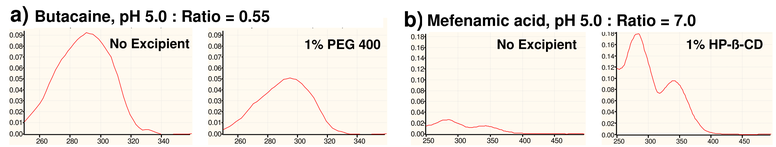

Fig. 2 a,b. Examples of case 2 (a) and case 3 (b) outcomes of the *in vitro* PK assay. Each plot pair shows OD in the acceptor compartment versus wavelength for no excipient (Control Plate, left) and excipient (Formulation Plate, right) setup.

Excipient Screening for Tamoxifen

Fig. 3 illustrates compound-excipient compatibility screening using the new method. A low soluble compound, Tamoxifen, was left alone (Fig. 3 a) or mixed with different excipients (Fig. 3 b-g) to select exci-pient(s) that would benefit absorption the most. 15 mM taurocholic acid and 1% HP-β-CD appeared to be best suited to improve flux of tamoxifen at pH 7.4 (see Fig. 3 c and d).

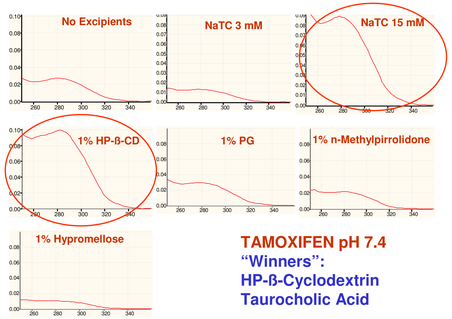

Fig. 3 Example of *in vitro* PK screening for the best excipient compatibility for tamoxifen. Presence of 15 mM of NaTau or 1% of HP-β-CD in the aqueous solution increases amount of permeated material more than 4 fold.

Simulated Intestinal Fluids

The *in vitro* PK method allows the effect of simulated intestinal fluids like FaSSIF/FeSSIF on absorption of drug compoundsto be studied. Fig. 4 shows effect of FaSSIF on absorption of Glybenclamide. Gain of about 2 fold can be seen for each of 12 measured wells.

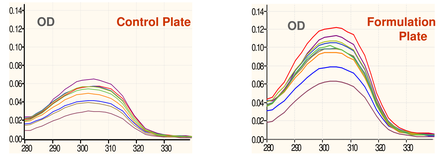

Fig. 4 Example of *in vitro* PK assay applied for screening of FaSSIF effect (Formulation Plate) on absorption of Glybenclamide. Each curve in the Control Plate and Formulation Plate represent a UV spectrum of acceptor compartment. Control Plate had SIF at pH 6.5 in the donor compartment while Formulation Plate had FaSSIF at the same pH.

Clinical Data Relevance

The predicted effect of HP-β-CD on absorption was compared to limited *in vivo* clinical PK data (courtesy of Hoffmann-La Roche). As can be seen in Fig. 5, *in vitro* data for 0.2% and 1% HP-β-CD correlates qualitatively well with *in vivo* clinical data shown as logarithm of C_{max} increase due to presence of HP-β-CD in the formulation. In contrast *in vitro* results for 5% HP-β-CD do not predict clinical PK data well.

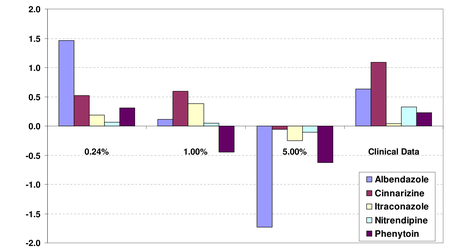

Fig. 5 Logarithm of FR in comparison with logarithm of C_{max} increase in clinical PK studies for a set of common drug molecules. Negative number means decrease in amount permeated, log FR = 1 means 10 times increase.

CONCLUSION

In vitro PK assay makes it possible to study the complex role excipients play in absorption while avoiding individual measurements of permeability and solubility, a task that is especially challenging for sparingly soluble compounds.

The new approach for excipient screening demonstrated the following advantages:

- Results are very *easily interpreted*
- *Fast turnaround* - only the acceptor spectra are read
- *Easy to set up* even for very low soluble compounds
- *No donor* and *no reference* need to be measured:
  compound can be very insoluble;
  excipient in donor can be turbid (e.g., FeSSIF/FaSSIF conditions);
  excipient UV absorbance will not interfere with results
- Reagents are validated in *Double-Sink™ PAMPA*
- *Inexpensive* compared to doing permeability & solubility experiments separately
- *Reagent kit & software* commercially available – *no development needed*

REFERENCES

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(2) Bendels, S.; Tsinman, O.; Wagner, B.; Lipp, D.; Parilla, I.; Kansy, M.; Avdeef, A. PAMPA-Excipient Classification Gradient Map. *Pharm. Res.*, **2006**, *23*, 2525-2535.
(3) Avdeef, A.; Kansy, M.; Nielsen, P.; Bendels, S.; Tsinman, K. Absorption-Excipient-pH Classification Gradient Maps: Sparingly-Soluble Drugs and the pH Partition Hypothesis. *Eur. J. Pharm. Sci.* **2008**, *33*, 29-41.
(4) Patent application, submitted jointly by *p*ION and F. Hoffmann-La Roche