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Review article

An integrated early formulation strategy – From hit evaluation to preclinical candidate profiling

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Abstract

The selection of a suitable vehicle for preclinical compound profiling is a very important task during the early developmental phases to ensure the quality of candidates and the speed of compound progression. Apart from biopharmaceutical and pharmaceutical technical considerations, i.e. solubility/dissolution improvement or route of application, other aspects have to be taken into account, as well: (i) availability and quality of the compound, (ii) tolerability of the vehicle in the selected animal model, (iii) developmental possibilities, i.e. whether the formulation can be transformed into a clinical one.

The approach described in this paper is based on results of team collaboration between functions involved in the conduct of animal experiments (Pharmacology, Pharmacokinetics, Toxicology, and Pharmaceutical Sciences). Very early *in vivo* studies should be performed with dissolved API as available information on solid-state characteristics is usually limited at this time. Later studies should be performed with developable formulations, taking into consideration pharmacological, toxicological, and pharmaceutical requirements. At this stage, delivery strategies (i.e. advanced formulations and/or alternative routes of administration) should be considered, as well. In addition, a minimum level analytical characterization of compounds and formulations used in animal studies is required to explain unexpected results.

Keywords: Early formulation strategy; Poor solubility compounds; Physical-chemical properties; Standard formulations; Enabling formulations; Harmonization; Customized vehicles

1. Introduction

The duration of drug developmental cycles are continuously challenged by an increasingly competitive environment. Due to modern high-throughput technologies, such as combinatorial chemistry and pharmacological screening, the number of new chemical targets intended for preclinical and clinical development has increased tremendously over the last few years [1]. To accelerate the selection process, more and more *in vivo* studies are conducted in parallel including those that (i) determine absorption, distribution, metabolism, and excretion (ADME)-parameters of a candidate per se, (ii) evaluate the compound's pharmacological effects, (iii) correlate its pharmacokinetic (PK) properties with its pharmacological effects (PK/PD studies), and last but not least, (iv) early safety studies that detect potential toxicological problems [2] (Fig. 1).

Despite the differences in objectives, sufficient and reliable exposure of the selected organism to the early-stage candidate is mandatory to obtain tangible results from these studies. Moreover, as target profiling is a multi-factorial optimization approach, comparable exposures derived from these studies are required for a successful candidate optimization and profiling.

Unlike late-stage development projects, early candidates are neither available in substantial quantities, nor do they have their manufacturing processes and physical quality

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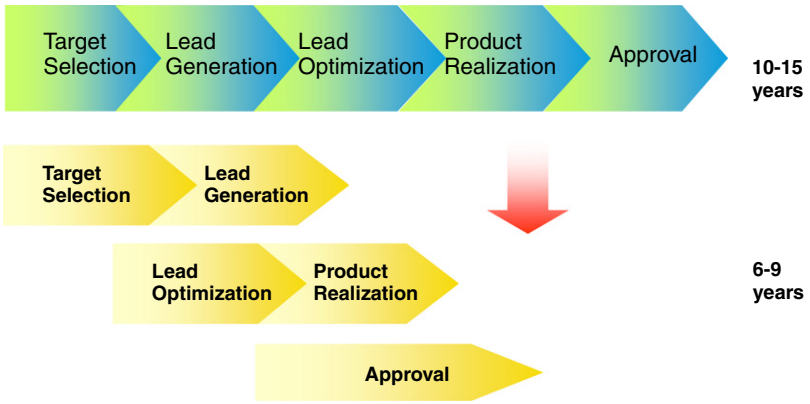

Fig. 1. Parallel working: change from sequential to overlapping value chain to reduce cycle time.

parameters optimized [3,4,9]. The latter facts lead to considerable variability in particle size and often also in crystalline state, hence the formulation system selected may also affect exposure.

In contrast to the past, when the majority of research compounds had a relatively small molecular weight and acceptable solubility, the number of larger and less soluble molecules displaying permeability- and/or solubility-limited absorption has increased tremendously during the last few years [5–7]. Therefore, it is not surprising that traditional formulation approaches, such as "disperse and dose", are no longer adequate in pharmacology laboratories as either the solvents required to dissolve hardly water-soluble compounds, or physical quality parameters of the early-stage candidates are likely to confound the biological results. Furthermore, the use of different formulation systems for the various *in vivo* studies in early development may lead to completely different exposures and hence inconclusive results.

Although the awareness of the relevance of physico-chemical drug parameters in compound development (e.g. "Rule of 5" and BCS [7,8]) has increased, preclinical formulation development needs to deal with poor compound properties, e.g. low solubility, more than ever. Consequently, scientists planning early *in vivo* studies need to address several challenges to allow fast and successful candidate profiling:

- Define strategies to select and provide well-characterized formulations yielding sufficient exposure and tolerability
- Define strategies to overcome drug delivery challenges due to unfavorable biopharmaceutical or physico-chemical properties of early candidates
- Define strategies to achieve optimum data comparability for different studies ("Harmonization/Standardization")

This paper describes a new approach to ensure optimum exposure in preclinical animal studies, while considering study-specific requirements, such as tolerability and limited compound availability, hence increasing the quality and speed of compound progression.

2. Preclinical formulations

While numerous publications have documented the significant efforts that have been invested into improving the throughput of screening technologies in chemistry, pharmacology, and in early stages of pharmacokinetic assessments [10], only a few addressed the many challenges associated with preclinical formulations [11,12].

Incomplete physico-chemical characterization, limited compound availability, and short timelines impair the development efforts already for "simple" formulations, such as solutions and suspensions. Another set of obstacles with potential effect on exposure is variations in physical quality parameters that need to be accounted for or better yet, compensated by preclinical formulations. Unfavorable compound properties, namely, poor solubility and poor permeability, further complicate the above difficulties and make the systematic development of enabling formulations even more necessary. In addition, conflicting requirements regarding formulation properties may force scientists to develop several different formulations for the various preclinical studies required to select an appropriate candidate for further development. A structured approach to efficient selection and development of preclinical formulations that targets all of the above-described challenges has been elaborated.

2.1. Efficient formulation development

Good intestinal permeability, as well as sufficient gastrointestinal dissolution are regarded as key factors in successful compound delivery following oral administration [8]. The permeability of a compound is a function of inherent determinants, such as molecular weight, ionized state (pKa), log D, H-bonding, and polar surface area [5,7,13–17]. It is a constant parameter under given pH conditions.

Efficient formulation development starts with assessing a compound's physico-chemical properties. These are typically available from high-throughput assays performed in chemistry departments or can be predicted using specialized software, such as ACD labs [18,13]. Classical decision

trees for discovery formulations, as described e.g. by Lee et al. [11], generally start with a specific formulation depending on the acid/ base properties of the compound in question and then iteratively optimize this formulation until e.g. a solution is obtained. However, by adding another level of information, it is possible to reduce the number of iterative cycles required for formulation development.

Based on a compound's aqueous solubility and pKa, its optimum solubility within a pH range of 1–8 can be easily evaluated and categorized [13]. By adding information regarding the compound's permeability, the necessity of developing a standard, intermediate, or an enabling formulation system can be readily assessed (Fig. 2).

Additionally, this solubility classification allows a first, experience-based feedback on the overall probability for the specific candidate compound to reach the clinical phase.

Depending on whether an acute or chronic application is targeted, this initial ranking is directly translated into a specific formulation system (based on a limited selection list) and serves as a starting point for developing the compound-specific formulation (Fig. 3).

In combination with very specific decision trees for each formulation system, this approach ensures a rapid and efficient formulation development.

Assuming that permeability is "beyond reach" for early formulations, it is evident that for small molecules with limited permeability ($P_{eff} < 10^{-6}$ cm/s) the solubility thresholds given in Fig. 2 need to be increased to achieve comparable overall flux.

Furthermore, adjustments to this general scheme need to be considered for e.g. highly lipophilic compounds (log $P > 5$), where particle size reduction does not lead to a sufficient increase in dissolution rate and absorption. In these cases, the development of alternative formulations, such as e.g. solid dispersions or lipid-based systems, might be considered the proper choice.

During a typical compound optimization and candidate profiling cycle, various animal studies with different target parameters are performed and often different formulations have to be developed – e.g. for acute vs. chronic applications.

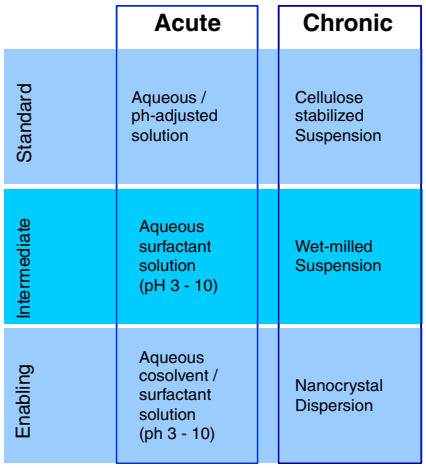

Fig. 3. Classified formulation systems for acute and chronic oral applications.

In those cases, the above described solubility-based classification can be linearly transferred: knowing that e.g. an "enabling" approach was required to develop a solution formulation indicates that a wet-milled suspension (intermediate formulation) most likely will not be sufficient to obtain a similar exposure. As shown in Fig. 4, only the development of a similarly classified formulation system, i.e. a Nanocrystal™ [19,20] dispersion, led to comparable exposure.

2.2. Formulation challenges

Although selecting the appropriate formulation system is already a major step ahead, there are still issues to address before the formulation can finally be administered to an experimental animal. The most prominent ones of these are the limited availability of API and short development timelines. This may be tackled by efficient formulation developments. However from a practical point of view, this also requires miniaturization of formulation technologies and standardization of formulation development.

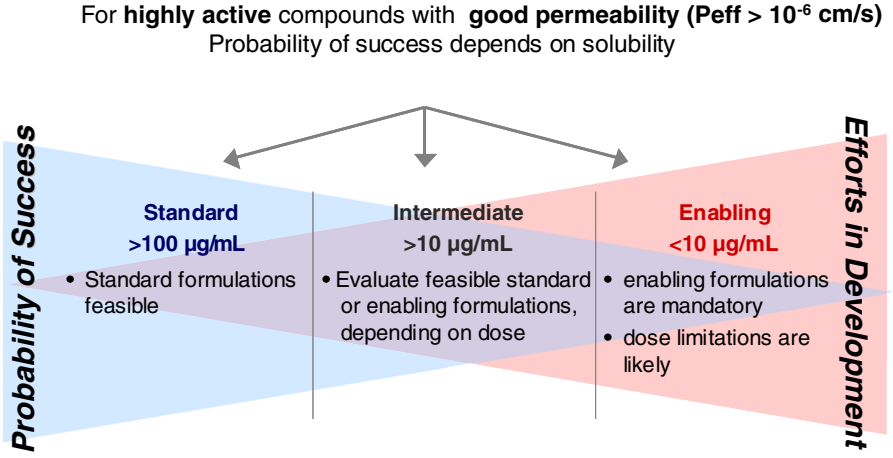

Fig. 2. Solubility classification for the selection of an appropriate formulation system.

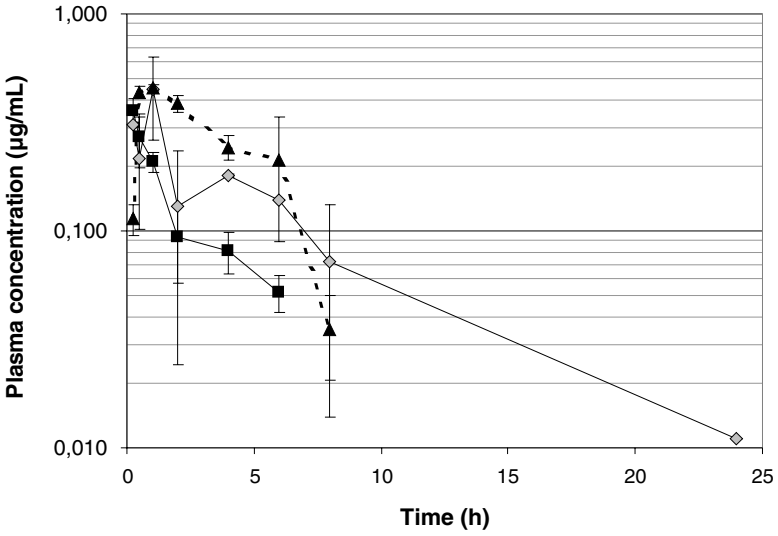

Fig. 4. Oral PK evaluation of a research compound in rat: co-solvent surfactant solution (20 mg/kg; —◇—) vs. wet-milled suspension (30 mg/kg; —■—) and Nanocrystal Dispersion (30 mg/kg; - -▲- -).

Miniaturization can be considered as a predominantly engineering task, which is relatively easy to solve for solution development but is more difficult a task when it comes to developing e.g. wet-milled suspensions or Nanocrystals™ [19]. Minimum drug substance requirements currently range from milligram quantities for solution development to 50–100 mg amounts for the development of enabling formulations.

Standardization of formulation development is a scientific challenge to the formulation scientist that calls for limitations in the depth of development, while it provides formulations with a reasonable robustness and scale-ability for clinical applications. By limiting the number of formulation technologies considered and by directing the choice of an appropriate formulation system as described in Fig. 3, it is feasible to limit experimental efforts to a maximum of 3–5 different composition variants for simple formulations and 5–10 different compositions for complex ones.

Another key issue in early formulation development arises from the early stage of chemical development: frequent changes in physical quality parameters of the candidates, such as particle size distribution, crystallinity, and morphic form. These parameters can affect solubility and dissolution rate and hence – in suspension type formulations – significantly influence PK properties as exemplified in Figs. 5 and 6.

Variations in particle size distribution can be minimized during manufacturing of suspensions by the use of standardization techniques, such as wet-milling. In contrast, addressing differences in crystallinity or morphic form would require considerable resources and more importantly, additional time in chemistry. As this would unacceptably slow down the candidate optimization process, the only way to manage changes in crystallinity is to characterize the solid-state properties of the API and of the API in

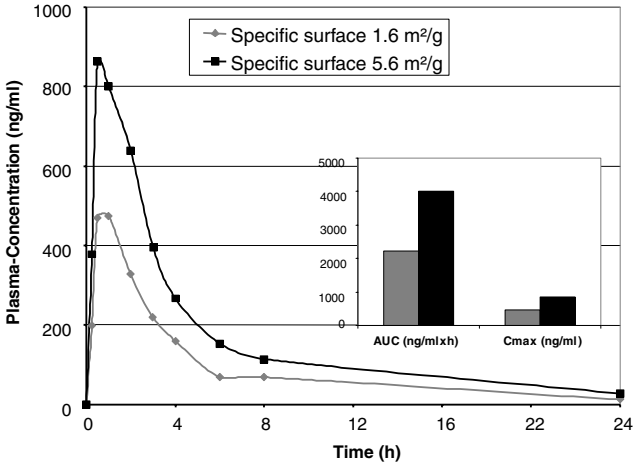

Fig. 5. Influence of a specific surface area on plasma concentration.

suspension. From this point of view, the use of solutions might be considered advantageous because the influence of the compound's solid-state properties on PK parameters is leveled out in this type of formulations. Unfortunately, potent co-solvents, such as e.g. *N*-Methyl-2-Pyrrolidone (NMP) and Diethyleneglycol-monoethyl-ether (Transcutol™), often display serious tolerability issues (e.g. drowsiness) in acute and/or chronic animal studies. Thus, the use of solutions vs. suspensions has to be carefully assessed for each study type (see Section 4).

3. Delivery challenges

Addressing and overcoming drug delivery issues caused by unfavorable compound properties, namely, poor solubility and poor permeability, is an important aspect in early development. Whereas exposure issues related to metabolic instability call for modifying the molecule (chemical

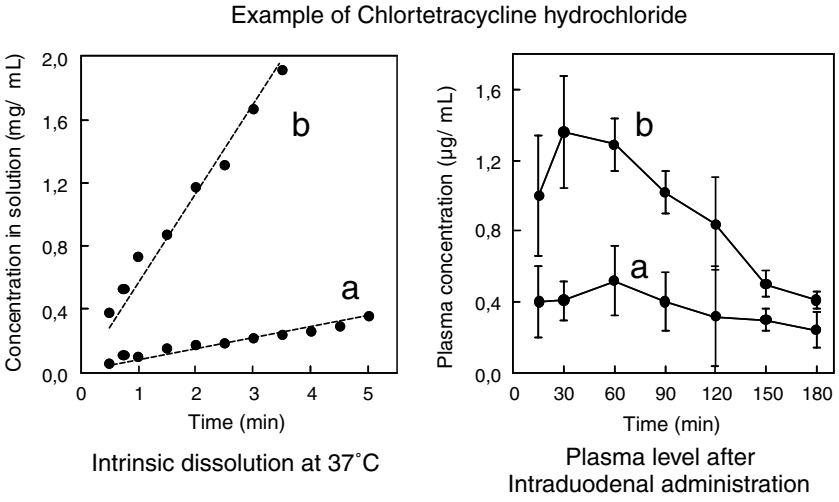

Fig. 6. Influence of polymorphism on plasma concentration; taken from [21].

optimization cycle), exposure issues caused by poor permeability and specifically poor solubility typically require the development of suitable ''enabling'' formulations early in discovery. There are basically three categories of enabling approaches, which are summarized in Fig. 7.

The classical enabling approach I (see Fig. 7) is clearly driven by solubility limitations: optimizing exposure to poorly soluble compounds has a long tradition in formulation development (e.g. use of air jet-milled drugs vs. unmilled material) and significant progress was made [22] pushing the solubility required to completely deliver even high doses of a compound to values in the low microgram to upper nanogram per ml range. Due to the huge dose range to be delivered to animals during the preclinical phase of drug development, mastering enabling technologies is a must for each formulation department. However, it is worthwhile to discuss the use of enabling technologies in compound optimization as it might be suspected that broad access of

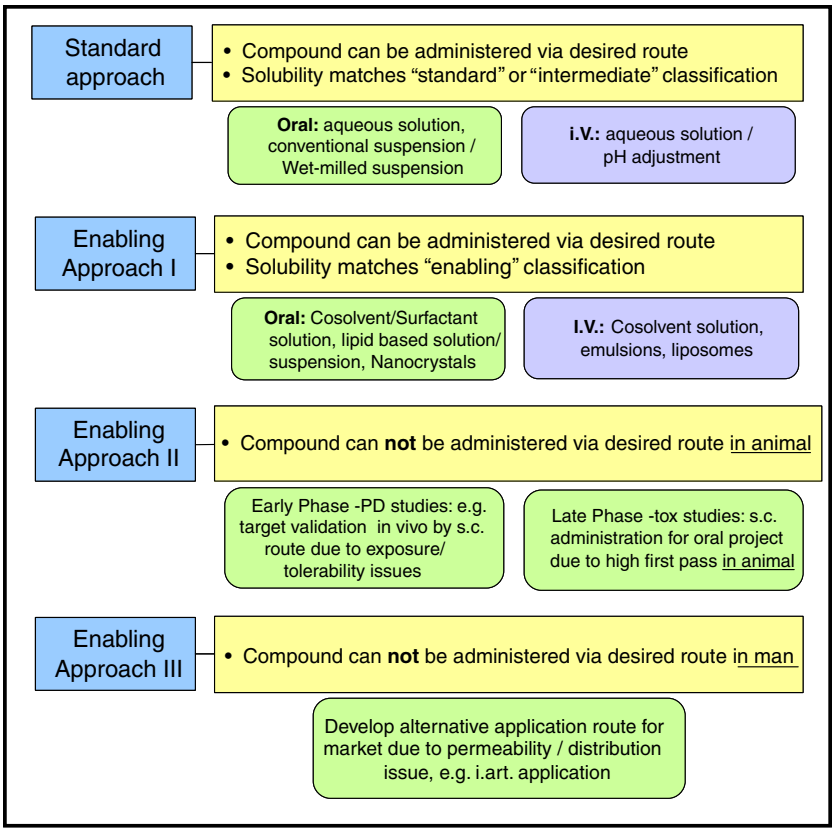

Fig. 7. Overcoming delivery challenges in early development (standard vs. enabling approaches).

discovery teams to those formulation technologies will trigger the development of even more poorly soluble compounds.

The approximately 10-fold increased demand for drug substances to be used solely for formulation development early in discovery is a strong incentive for teams to focus their efforts on compounds with better solubility. In our current practice, enabling formulations for poorly soluble compounds are typically used either

(a) as means to evaluate the pharmacological activity of a compound series on a specific target (target validation), while chemical optimization is continued aiming at effective compounds with improved solubility or permeability
or
(b) as final formulations when a chemical series cannot be further optimized and the respective candidates are highly effective on a specific target.

Enabling approaches II and III are predominantly driven by permeability or metabolic issues. When a promising compound does not reach the systemic circulation or specific tissues/compartments after oral application, alternative routes of administration, like intravenous subcutaneous, intramuscular, pulmonary, etc., should be considered as means to overcome limitations for specific preclinical studies (enabling approach II) or as a rescue approach to develop a marketed formulation (enabling approach III). However, as oral application is by far the most popular route of administration, lower compliance by physicians and patients for all other routes of administration may be expected unless the medical need is extremely high.

Another theoretical option to overcome poor permeability for BCS classes III and IV compounds would be the co-administration of absorption enhancers [23–25]. This approach bears considerable risks of toxicological issues (many of the absorption enhancers have toxicological and pharmacological side effects, specifically at long-term treatment) and development issues (co-development of the absorption enhancer and the compound is required). In spite of these negative features, this concept is being pursued from time to time, e.g. for oral delivery of peptides, such as insulin, calcitonin, etc.

In early development, the elaboration of permeation enhancing formulations is hardly applicable as general strategy due to the increased resource requirements associated with such development but it might be considered in specific cases. Furthermore, this concept appears to have no proven track record of successful launches for oral application so far, and for this reason, it is not included in Fig. 7.

4. Harmonisation/standardization in preclinical studies

In most pharmaceutical companies, the determination of ADME-parameters has become both a selection criterion and an optimization parameter in the early value chain [26]. Consequently, the DMPK departments try to evaluate the PK behavior of compounds as early as possible using a battery of *in vitro* and *in vivo* assays. Typically, the process starts with various *in vitro* models (e.g. PAMPA, Caco-2, S-9 metabolic stability, metabolism by hepatocytes, cytochrome P450 interaction, protein binding, etc.) resulting in the selection of a reduced number of candidates to be subsequently tested *in vivo* [27]. Virtually all *in vitro* tests are performed (and can only be performed) with solutions, whereas *in vivo* studies can be performed both with solutions and suspensions.

4.1. Formulations for PK screening in vivo

The preferred formulation system for early PK studies needs to be suitable for single and multiple compound dosing, while allowing the candidates to display their inherent PK properties, such as absorption rate, clearance, etc., without enhancing them. Although solutions require some developmental efforts, specifically for highly insoluble compounds in cassette dosing settings, they are generally preferred, as the influence of the compound's solid-state properties on PK parameters can be neglected. Of course, this has to be balanced with two potential disadvantages of solutions when compared to suspensions: the risk of compound precipitation during intestinal transit and potential changes of PK parameters by the co-solvent(s) used.

For this reason, it is a key element of the described Early Formulation strategy to use solutions for all PK study designs (single administration or cassette dosing, if applicable) and to mitigate the associated risks by

- evaluating the precipitation tendency of solutions by dilution with simulated artificial media (SGF/SIF/Fassif/Fessif [28])
- selecting appropriate vehicles with no or only small effects on the PK parameters of compounds and selecting one solvent system for all studies within one chemical series (COP).

The latter minimizes any remaining effect of the selected vehicle on the PK parameters of the early candidates and allows unbiased ranking within one series. It has to be noted that in an industrial environment, this is not always feasible given the tight timelines in early projects and the iterative nature of compound optimization. However, selecting robust formulations for the very first compounds within a chemical series increases the chance to operate at minimum within a narrow range of solvent systems.

Based on internal experiences, the recommended solvents for early development stages [29] are listed in Table 1.

4.2. Formulations for PK profiling studies

The results of early *in vivo* studies using solutions yield information on the PK-properties of early candidates *per*

Table 1
Preferred solvents for early *in vivo* animal trials; taken from [29]

Standard solutions for oral administration
Aqueous standard solutions
→ Acidic or basic aqueous standard solutions
Aqueous co-solvent standard solutions* (*Co-solvents*: Polyethylene glycol, propylene glycol, glycofurol 75, glycerine, ethanol, transcutol); amount depends on safety aspects /drug concentration required
→ Acidic or basic co-solvent standard solutions
Micellar solutions for oral administration
Aqueous surfactant solutions (*Surfactants*: Labrasol, Polysorbate 80 (Tween 80), Cremophor RH 40; Lecithins); amount depends on safety aspects/drug concentration required
Aqueous co-solvent/surfactant solutions*
Mixed micellar solutions
Fatty oil solutions for oral administration
Pure fatty oil solutions
Fatty oil/surfactant solutions
(Emulsions)

Aqueous phases of mentioned systems might be isotonic, pH adjusted (slightly acidic or basic) or slightly buffered Advanced systems to be evaluated on a case by case basis. * Most frequently used in early animal studies due to easy production.

se and allow a ranking of compounds within a series. However, these data often do not provide enough information on the developmental prospects of a compound. Therefore, in a next step, it is often required to obtain the first data about the exposure of animals using a suspension formulation. These studies can be used

(i) to assess and predict the exposure of animals in upcoming toxicology studies, which are often conducted using suspensions,
(ii) to assess the exposure of animals after administration of higher doses, and
(iii) to assess the candidate's suitability to be delivered from solid dosage forms.

For these studies, the characterization of the physical quality attributes of a compound and its suspension(s) should be performed prior to their use. Furthermore, quality data for the suspension(s) finally applied to animals need to be generated: i.e. content, impurities, crystalline form, amount dissolved, etc. By these measures, it is possible to reduce the risk of obtaining irreproducible exposures and study results that are not comparable.

4.3. Flowchart for PK studies

A possible process for biopharmaceutical profiling is summarized in Fig. 8: Starting with solution formulations either in a cassette, or in single administration approach in rats and dogs, first pieces of information on the PK parameters of the compound are obtained. Insufficient exposure usually results in the search for new, improved compounds, which is often combined with activities that identify the reason for the low exposure. If compounds with excellent exposure after administration in solution are identified, studies with characterized suspensions are initiated. By preference, two different doses are evaluated to obtain the first information on dose linearity of the PK parameters. When a compound displays excellent exposure after administration in solution but poor exposure after dosing as a suspension, physical quality attributes are modified (e.g. milling of the compound, production of nanoparticles, etc.) to improve exposure. Only those compounds that display acceptable PK parameters both after administration in solution and suspension qualify for further development.

4.4. Pharmacology studies

With regard to design and target parameters, pharmacodynamic (PD) studies are completely different from DMPK-studies (Table 2). Due to the more complex

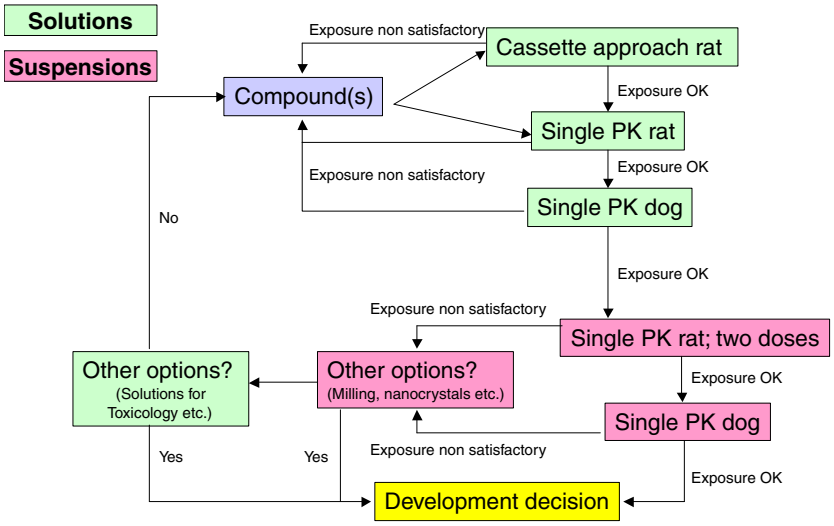

Fig. 8. Flowchart – example for biopharmaceutical profiling.

Table 2
Differences between PK- and PD-studies

PK-studies	PD-studies
Cassette dosing feasible	Cassette dosing impossible
Single administration	Often chronic administration
PD-effect of solvent negligible	PD-effect of solvent not acceptable
Weak site effects acceptable	Also weak site effects not acceptable
Normal animals	Special animal models
Healthy animals	Often diseased animals
Evaluation rapid	Evaluation more complex
Repetition possible	Repetition sometimes impossible
No vehicle control	Vehicle control groups required
No parallel PD-effect analysis	Parallel PK-analysis urgently required

character of PD studies and specifically to their longer duration, these studies need to be exploited to an optimum to make best use of limited resources and to shorten development times. To allow this, it is mandatory to consolidate the contributions of a panel of scientists from different functions already in the planning phase of PD studies: The study owner and leading scientist in the study conception and conduct should be the pharmacologist. He needs to be supported by a DMPK-scientist to define optimum time points to collect blood, plasma, or other body fluids and hence to make sure that reliable data regarding animal exposure will be available allowing the establishment of PK/PD-relationships. In addition, a toxicologist's expertise is required to define critical organs and tissues to be collected during and/or after the study. Finally, formulation scientists are required to define the best-suited formulation, that yields sufficient exposure, while avoiding or minimizing any excipient-related effect on PD parameters.

In summary, optimal PD studies require

- A team-based study planning by scientists from the Pharmacology, DMPK, Toxicology, and Pharmaceutical Departments
- A vehicle-control group to exclude potential PD effects of the formulation *per se*. For example, animal models used for rheumatic diseases or osteoarthritis have a long track record of vehicle-induced changes in PD parameters.
- The analytical characterization of any study formulation
- The determination of the exposure of the animals to generate early PK/PD relationships.

For established animal models, which are frequently used, the definition of a set of standard formulations can help reduce the number of animals as control groups may be omitted when sufficient prior experience is available. An example is given in Table 3.

However, due to the differences in animal models (species, subtype, etc.) and to the variety of target parameters, preclinical formulations will often be specific for certain disease areas, such as e.g. cardiovascular or metabolic diseases, arthritis, etc.

Table 3
Formulations proposed for cardiovascular studies (selection dependent on animal model)

Suspensions, oral – for long term treatment
0.5% Hydroxyethylcellulose, wet milled
0.5% Hydroxyethylcellulose, wet milled, including 0.1% Tween 80
0.5% Hydroxyethylcellulose, wet milled, including 50% Lipofundin N 20 (10% soy bean oil in mixture)
Nanocrystals
Solutions oral
20–50% PEG 400
20–50% Glycofurol 75/ Cremophor (3 + 1) in saline or buffer.
Phosal 50 PG – mixture of 50% Propylene glycol and 50% soy bean lecithin
Tween 80 (up to 10% in water)
Hydroxypropyl–betacyclodextrin (up to 30%) in water
Labrasol/Gelucire (1 + 1) – diluted with 50–90% water or buffer (emulsion)
Miglyol/Lecithin (95 + 5) – tb homogenized with 50–90% water (emulsion)
Solutions, i.V.
20% PEG 400 in saline, 0.2 mL/kg (inject slowly)
50% PEG 400 in saline, 0.1 mL/kg (10 min infusion)
20–50% Glycofurol 75 in saline.
20–50% PEG/Glycofurol/Poloxamer 188 (39/10/1), in saline or buffer
20–50% Glycofurol 75/ Cremophor (3 + 1) in saline or buffer.
20–50% Glycofurol 75/ Solutol HS 15 (9 + 1), in saline or buffer
Caveat: Surfactants may cause release of histamine, if administered via parenteral route

4.5. Toxicological studies

In terms of formulation standardization, toxicology departments are the most advanced among the preclinical functions. To avoid possible interferences of excipients with drug-related effects in long-term studies, toxicological studies are conducted by preference with suspensions stabilized with cellulose derivatives, such as e.g. methylcellulose or hydroxy ethyl-cellulose. This allows to bridge actual study results to a wealth of data consolidated over decades. Nonetheless, it is important to make sure that toxicology studies are considered as part of the overall set of preclinical animal studies. To ensure comparability with PK and PD studies, it is required that

- The formulation has been analytically characterized
- Sufficient exposure of the relevant species after administration of an identical formulation has previously been demonstrated
- Dose-linearity, at least for lower dose levels, has been demonstrated.

In case exposure from standard suspensions is insufficient, either due to poor solubility in the gastrointestinal tract, or poor PK properties (e.g. permeability, first pass metabolism), specific formulation efforts are

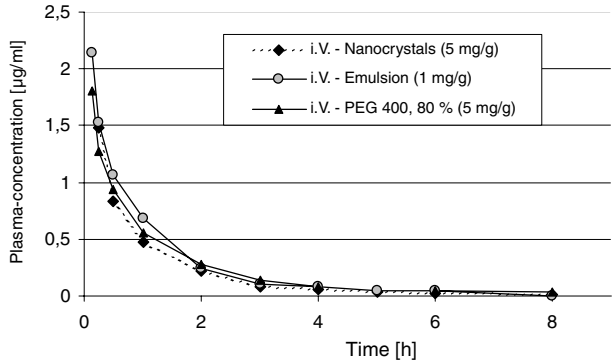

Fig. 9. Overcoming first-pass metabolism and tolerability issues by use of enabling strategies. Poorly soluble compound (<0.5 µg/mL) was given intravenously (IV) to dogs at a dose of 2.5 mg/kg as nanocrystal formulation, as PEG-solution, and as emulsion. (I) Exposure of compound could be tremendously increased compared to oral application (C_{max} at 200 mg/kg <0.5 µg/mL, data not shown). (II) Local tolerability issue of very poorly soluble compound after IV application in solution could be handled by use of nanocrystal – or emulsion formulation w/o significant change of PK profile.

required to avoid delays in the development program. Typically, solubility-limited BA issues can be addressed by developing enabling formulations like nanoparticulate systems or lipid-based delivery systems [30]. Permeability and metabolism-linked exposure issues can be overcome by a change in the route of administration, e.g. i.v. instead of oral application (see Figs. 9 and 7).

This approach can also be considered for early validation of targets during pharmacological profiling in case early compounds do not show favorable PK properties after oral application (Fig. 7).

Naturally, the expected increase in exposure should be demonstrated before any PD study starts. Apart from this, excipient tolerability should be considered to avoid formulation-related side effects in long-term studies.

5. Summary and discussion

Establishing an early formulation concept is key to increase the quality and speed of compound progression. The presented approach ensures optimum exposure in preclinical animal studies, while considering study-specific requirements, such as tolerability and constraints in resources and compound availability. Efficient formulation or in other terms, pre-selection of appropriate formulation systems based on solubility and pKa data, is crucial to reduce the number of experimental iterations in formulation, as well as to obtain formulations yielding sufficient exposure. Formulators need to overcome challenges due to limited availability of drug substance through miniaturization and standardization of formulation development in order to achieve the aspired goal.

Addressing delivery issues caused by poor solubility or poor permeability of candidates is still a considerable obstacle in compound optimization. For poorly soluble drugs, the availability of enabling formulation techniques already during early discovery that are transformable into clinical formulations is a prerequisite. However, the advantage of quickly progressing with an enabling formulation needs to be balanced with the additional efforts required in later development stages caused by the more complex formulation (Fig. 2).

Poor permeability is less likely to be addressed by formulation technologies but requires a different route of application or chemical optimization. Highly specific, enabling formulations are without value or even detrimental to rapid project progress when inappropriately used. For this systematic approach, selection of formulations for all kinds of preclinical animal studies is required. Teamwork and excellent cooperation between the involved functions (Pharmacology, DMPK, Toxicology, and Pharmaceutical Departments), a continuous knowledge exchange and the ability and willingness to exploit study results to a maximum are mandatory.

In the interest to achieve an optimum comparability of *in vivo* data in the early development phase, the contributing functions have to select the formulations they use in a more careful way than they did in the past. This can be accomplished by adhering to the following major rules:

DMPK	Use solutions whenever possible for early studies; re-confirm results using *characterized suspensions.*
Pharmacology	Discuss study design with other functions before starting the study. If possible collect PK-samples from the same set of animals simultaneously (PK/PD); conduct all PD-studies with a vehicle control group.
Toxicology	Consider alternative formulations and/or alternative routes to establish no observed adverse effect levels if suspensions do not work. If using suspensions, characterization is mandatory.
Formulation	Provide early formulations that are transferable into marketed dosage forms.

Furthermore, this approach decreases the number of animal studies and consequently contributes to the "3R"-approach for animal trials (reduce, replace, refine). It also shortens development times and leads to lower costs.

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