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Impact of dosing schedule in animal experiments on compound progression decisions

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Low pipeline output is a major problem for the pharmaceutical industry. Many compounds fail to enter clinical development, owing to toxicities in animals. Using pharmacology principles, this paper argues that the often-infrequent dosing in animal experiments despite a drugs short half-life produces a large fluctuation in drug concentration, which can severely erode the therapeutic index and safety margin for the anticipated clinical dose, consequently preventing potentially effective treatments from entering human trials. Aided by computer simulations under generalised conditions, the analysis was conducted in a quantitative framework with broad relevance. The findings call for more-translatable dosing practices through closer collaboration among scientists in multiple disciplines and better regulatory guidance for animal studies to better inform compound progression decisions.

Preclinical□clinical attrition: where have all the good drugs gone?

The viability of an investigational drug is largely determined by its efficacy potential and its safety risk. In current drug development paradigms, important decisions for a clinical programme are often guided by findings from animal experiments. A good therapeutic index (TI), which is the ratio between the amount of the drug that is harmful and the amount that is therapeutic [1], estimated from animals, is required for progressing a discovery_stage asset into preclinical development through candidate selection (CS). An asset in preclinical development enters a clinical programme through the first-in-human (FiH) trial if the safety margin (SM) for the predicted human therapeutic dose in relation to the no-observed-adverse-effect level (NOAEL), which is the highest amount of the drug that does not cause more harm than vehicle control in the most sensitive relevant toxicology species, remains sufficiently high [2,3]. In practice, the required level of this safety coverage depends on the nature of the unmet medical needs, the severity of the dose-limiting toxicity and whether the toxicity is monitorable and reversible.

It is recognised across drug_iscovery organisations that >85% of lead compounds proposed for CS [4] or 36% of those having passed CS [5] do not enter FiH. This high attrition has many causes, including: new evidence on the target such as insufficient role in the disease or inherent safety risk; unsuitable physical, chemical or biological properties of the molecule causing insufficient penetration to target site or insufficient target binding; high cost or insurmountable obstacle for development or manufacture; and lack of commercial viability or competitiveness. But the primary reason for 59% of the terminations was toxicity [4], implying insufficient TI or SM as a key driver in many cases.

Compounds that are considered for CS normally have high *in vitro* potency in the nanomolar or picomolar range, and high selectivity of hundreds or thousands of folds against a large screening panel of proteins. This leads to the question: how can so many highly potent and highly selective molecules lose TI or SM before FiH? Screening panels cannot possibly include all known enzymes or receptors; plenty others,

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known and unknown, could contribute to *in vivo* toxicity. The translation from *in vitro* screening to an *in vivo* system is often poorly understood for wanted and unwanted activities, so the *in vitro* potency and selectivity might not translate into *in vivo* efficacy and safety. And, there are other factors at play. One possibility is that inadequate dosing frequency used in toxicology studies, or proposed for human trials, contributes to the erosion of TI and SM. To test this hypothesis, virtual experiments for a hypothetical drug have been conducted by computer simulation as described below; their results suggest that inadequate dosing frequency could indeed be a key culprit. Although the importance of translatability for animal experiments and pharmacokinetic difference between species are broadly recognised conceptually, dosing regimens in animal experiments are often suboptimal for addressing these issues in practice. The intent here was to use simulation to illustrate the magnitude of the issue. For simplicity, the simulations were conducted under steady-state conditions for a systemic drug requiring regular (i.e., chronic) administration. This reflected the therapeutic scenario for most investigational drugs, and the findings could be expanded in principal to topical treatments.

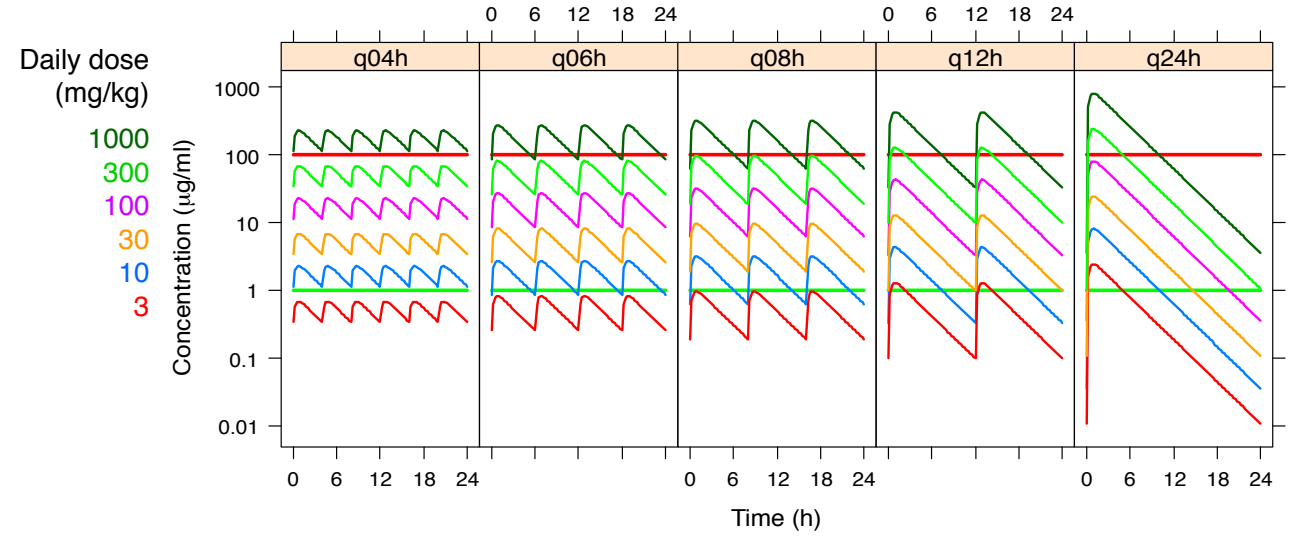
The hypothetical drug

Drugs produce wanted and unwanted responses through their concentrations. In theory, true TI can be defined as the ratio between a maximal safe concentration and a minimal effective concentration; in practice, the TI is estimated as the ratio between the maximal safe dose and the minimal effective dose. For a hypothetical drug that has a true TI of 100, for an animal species and in human, the time course of its concentration was simulated for both species at a range of doses, each administered per several regimens.

For simplicity, the hypothetical drug had first-order absorption, first-order elimination and one compartment distribution in both species. The apparent volume of distribution and apparent clearance were scaled over bodyweight across species using the widely accepted allometric coefficients of 1 and 0.75, respectively [6,7]. Both species had an absorption rate constant of 3 h^{-1} and apparent volume of distribution of 1 l/kg. For the animals, in this case rats with a bodyweight of 0.2 kg, the clearance was 0.0479 l/h. For humans with a bodyweight of 70 kg, the clearance was 3.87 l/h. Consequently, the half-life was 2.9 h in the animals and 12.5 h in humans. Although the exact values for these parameters were not important for this analysis, they were well within the ranges of usual values observed for the respective species in drug programmes, hence were realistic choices.

Therapeutic index in the animals

The simulated concentrations for the animal species are depicted in Fig. 1, where daily doses from 3 to 1000 mg/kg were either given as a single administration or split at intervals from 4 to 12 h. As dictated by linear pharmacokinetics principles, the exposure increased with dose and remained constant for the same daily dose regardless of the regimen. As expected, less frequent dosing produced more-fluctuating concentrations: the peak:trough ratio increased from 2.0 to 13 when dosing changed from



Dosing frequency	Every 4 hours	Every 6 hours	Every 8 hours	Every 12 hours	Every 24 hours
Peak:trough ratio	2.0	3.2	5.1	13	230
MED (mg/kg)	10	30	30	30	300
NOAEL (mg/kg/d)	300	300	300	100	100
Calculated TI	30	10	10	3.3	None

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FIGURE 1

For the same daily dose, less frequent dosing produces more-fluctuating concentrations, subsequently increasing minimal effective dose (MED) and reducing no-observed-adverse-effect level (NOAEL) and therapeutic index (TI). Green and red straight lines depict the minimal effective concentration and the maximal safe concentration, respectively. Only doses with a concentration profile entirely contained between these two lines are considered safe and effective.

six-times daily to twice-daily, and to 230 at once-daily. For simplicity, assuming only doses with a concentration profile entirely contained between the maximal safe concentration and the minimal effective concentration are safe and effective, the estimated TI decreased from 30 for six-times-daily dosing to 3.3 for twice-daily dosing, and completely disappeared for once-daily dosing.

No universal agreement on acceptable TI exists or should exist. Whether a TI is acceptable depends on a drug□s benefit:risk ratio which in turn relies on the intended indication, the severity, detactability and reversibility of the toxicity, and the availability of alternative treatments. To my knowledge, once-daily dosing in preclinical experiments is not uncommon among companies; neither are terminations for TI <10. This example of a potentially useful drug with a true TI of 100 would probably fail CS in many situations if the animals are dosed less frequently than three-times-daily. The simulations suggest that potentially good drugs might have been discarded, owing to inappropriate dosing.

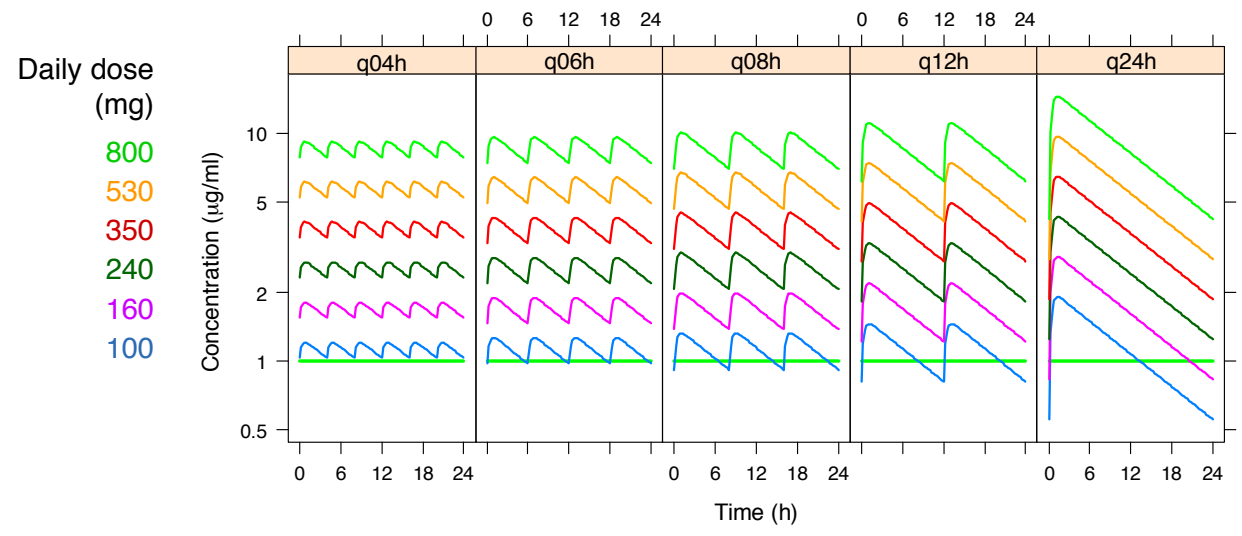
Whenever there is concentration fluctuation, TI expressed as a dose ratio is lower than expressed in a concentration ratio. However, less _frequent dosing can cause the (peak) concentration to become toxic at a relatively low daily dose which produces a relatively low overall exposure and low (trough) concentration that fail to maintain efficacy. In this case, the NOAEL shrunk from 300 mg/kg/day for three-times-daily to 100 mg/kg/day for twice-daily dosing, directly affecting SM calculation for the anticipated human therapeutic dose.

Safety margin for humans

Drug concentrations were simulated for humans at a range of doses administered per various regimens, to calculate the SM for anticipated minimal therapeutic dose. Per regulatory guidance and usual industry practice, the SM was calculated as the ratio for maximal concentration (C_{max}) and total exposure (area under the concentration time curve; AUC), between the NOAEL for the animals and the anticipated minimal therapeutic dose for humans [2,3]. For simplicity, the minimal therapeutic dose for each regimen was defined as the lowest dose with its concentration curve entirely above the minimal effective concentration.

The minimal therapeutic dose increased when dosing became less frequent, for example from 160 mg/day for twice-daily to 240 mg/day for once-daily (Fig. 2). Importantly, less frequent dosing in the toxicology experiment and less frequent dosing proposed for human trials, each alone and in combination, eroded SM owing to lower NOAEL and higher minimal therapeutic dose. For example, when dosing was four-times-daily in the animals and proposed to be twice-daily in humans, the SMs were good at 37 and 31 for C_{max} and AUC, respectively. By contrast, at twice-daily in the animals and once-daily in humans, the corresponding SMs dropped to 10 and 7.

As for TI, the acceptability for SM is a matter of case-by-case judgement depending on the drug□s benefit:risk ratio. An informed decision relies on shared understanding among biologists, toxicologists, drug disposition scientists, clinical pharmacologists and clinicians. A severe erosion of SM at best limits the dose range in



	Animal dosed every 6 h (NOAEL = 300 mg/kg/d)		Animal dosed every 12 h (NOAEL = 100 mg/kg/d)	
	Cmax	AUC	Cmax	AUC
Proposed twice-daily in human (predicted MThD = 160 mg/d)	37	31	20	10
Proposed once-daily in human (predicted MThD = 240 mg/d)	19	21	10	7

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FIGURE 2

Less frequent dosing used in toxicology studies and proposed for human trials reduces estimated safety margin (SM) between no-observed-adverse-effect level (NOAEL) and the minimal therapeutic dose (MThD), defined as the lowest dose with a profile entirely above the minimal effective concentration, in terms of maximal concentration (C_{max}) and total exposure (AUC). Green straight line depicts the minimal effective concentration.

clinical trials and at worst prevents the drug from being tested in humans altogether. With a single-digit SM, this hypothetical drug probably would not progress to FiH because the SM values would not be sufficient for many indications, despite the true TI of 100.

Implications of the findings

Two observations motivated this analysis: highly potent and highly selective compounds approaching CS are often terminated owing to safety concerns before clinical testing; and drugs are usually dosed infrequently in toxicology experiments despite their short half-lives. The findings reveal that infrequent dosing in toxicology experiments could severely erode the TI in animal species and the SM for anticipated human doses, potentially causing ill-informed programme terminations. The SM would be further worsened when infrequent dosing is planned in clinical trials, with the expectation of better patient acceptance and therapy adherence.

The most important premise for this analysis is that the concentration needs to be contained between a minimal effective level and a maximal tolerated level, to produce meaningful pharmacology without unacceptable toxicity. Although this can seem arbitrary and simplistic, because strictly speaking all concentrations however large or small on the concentration curve produce pharmacology and toxicity, it is consistent with the sensible early-phase drug development practice of maintaining adequate trough concentration (e.g., above EC_{50} or EC_{90}) and suppressing the peak concentration (e.g., below one-fifth of NOAEL). Although conditions reflecting alternative practices could have been used, such as keeping the concentration profile within a certain range for a sufficient duration (e.g., above EC_{50} for at least 90% of dosing interval and above EC_{90} for no more than 10% of dosing interval), these are just variations reflecting the same principle despite being more-complex but equally arbitrary.

To ensure general relevance and for simplicity, a framework of direct links between drug concentration and response was used in the simulations. Drug effect need not link directly to the circulating concentration: a pharmacokinetic–pharmacodynamic disconnect occurs if drug access or binding to a target takes time [8], or if the drug alters the production or clearance of cells, enzymes or receptors and a meaningful change in the species' level takes time to establish [9]. The greater this disconnect is, for efficacy or toxicity, the less impact the fluctuation of circulating concentration has on TI estimation, and the less sensitive the TI estimation is to dosing frequency. For simplicity, the analysis also assumes the pharmacokinetics to be linear in both species, whereas absorption or elimination can in reality become saturable at higher doses, reducing the peak:trough concentration ratio. When this happens to a greater extent in animals than in humans the erosion of TI or SM would be less severe. In addition, a faster absorption rate in the animals would cause greater erosion, and vice versa.

To test the hypothesis with experimental data, dose-ranging toxicology studies would need to be run for several compounds in a sufficient number of animals at multiple dose levels for a range of dosing frequencies – costly experiments that are difficult to justify for compounds in development and more so for those already terminated. Therefore, simulations were conducted using a hypothetical drug with realistic properties to quantitatively and systematically test this hypothesis. Nevertheless, it would be interesting to see whether development of historical compounds with higher pharmacokinetic fluctuation was indeed terminated more often owing to toxicity, provided that relevant information including a primary termination decision was adequately documented.

Despite the absence of comparative experimental data, multiple factors lend credibility to the principle findings of this analysis. The premise that drug concentration drives effect is a fundamental pharmacological principle; the assumption that the window between a minimal effective concentration and a maximal safe concentration determines a drug's TI is sound and applied fairly to all regimen scenarios; the scaling relationship between animal and human is well established; the half-lives in the respective species reflect common observations; and the values of the derived safety parameters (TI, NOAEL and SM) driving progression decisions are realistic.

Designing appropriate dosing schedules

A dosing schedule should reflect the nature of the target pathway, the properties of the compound and the mode of drug–target interaction. Adequate dosing frequency depends on factors such as the dynamic range of effective concentrations, ease of access to the site of action, presence of active metabolites, target modulation mechanism and rate-limiting step of the transduction pathway. Drugs acting at the steep part of the pharmacodynamic curve are more sensitive to concentration fluctuation; drug concentration at the effect site will fluctuate less if site penetration is slow; active metabolites can prolong drug action; irreversible inactivation of a slow-recovering target requires less frequent dosing; a rate-limiting step downstream in the transduction pathway usually means a less sensitive response to dosing frequency; and intermittent pharmacokinetic profiles could even be desirable in certain situations to avoid tolerance development or to create drug holidays for better tolerability. The importance of a sufficient half-life in compound design and selection, to limit peak:trough concentration ratio for ensuring pharmacology and limiting toxicity, has been well illustrated in recent literature [10,11].

Moreover, dosing schedule decisions should not be solely based on the anticipated and desired pharmacological effects. Recognising the possibility of (unexpected) off-target toxicities is also important. A high peak:trough ratio risks producing toxicity while maintaining pharmacology. Hence, in principle, it is generally prudent to keep the peak:trough ratio within a reasonable range unless there is sound justification not to. Unfortunately, the guidance for conducting nonclinical safety studies to support human trials, published by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, does not provide views on dosing schedule requirement [12]. This is an important area that urgently requires improvement through collaborative efforts by drug developers and regulators. Dosing schedule in toxicology studies should aim to reflect the anticipated exposure profile in clinical trials for relevance and translatability.

Enabling animal–human translation

Evaluating the potential TI before clinical testing is highly complex and situational. Pharmacological assessments could include a range of *in vitro* tests with human targets or cell lines, human or animal tissues or organs, and *in vivo* tests in animal disease models. Similarly, safety assessments, for general and specific (e.g., cardiovascular, neurological, reproductive and mutagenic) toxicity could also include a variety of *in vitro* and *in vivo* tests in relevant species with adequate levels of target expression. The collective data on efficacy potential and safety risk grow, and the knowledge basis for predicting TI evolves, when the asset approaches CS and moves towards FiH. Extrapolating TI from animals to humans and calculating SM usually also requires cross-species pharmacodynamics

(e.g., potency and target expression) and pharmacokinetics (e.g., plasma tissue binding and tissue partitioning) corrections.

The usefulness of animal experiments conducted to infer human efficacy and safety hinges on the translatability of results. The lack of animal□-human efficacy translation has long been recognised as a major obstacle in drug development, raising the importance of effective use of relevant biomarkers for in-depth mechanistic understanding of species difference [13]. This is equally important for safety translation, because efficacy and toxicity both result from the drug□s action on a biological system. This has been extensively analysed [14], and regulatory guidance increasingly emphasises the importance of mechanistic understanding of safety findings [12]. However, the knowledge of a drug□s toxicology is usually limited, especially for off-target effects as opposed to on-target ones, which are often exaggerated pharmacology. As such, the application of TI, NOAEL and SM for extrapolating safety findings from animals to humans remains largely empirical.

Despite these challenges, it is important to maximise translatability through known factors such as drug half-life or controllable variables such as dosing schedule. A common argument for once-daily dosing in toxicology studies is to reflect the aim for once-daily dosing in humans. Dosing a drug to animals (with a half-life of 3 h) once-daily (or every eight half-lives) resembles dosing the same drug to humans (with a half-life of 12 h) every four days. Figure 3 illustrates that, when the animals and humans are dosed once-daily, the concentrations in the animals would be far more fluctuating and with a far higher peak concentration for the same total exposure. This is consistent with the observation that the safety margin for a human dose is usually higher in terms of the C_{max} than in terms of AUC; the high peak at the animal NOAEL limits the potential for achieving effective exposure in humans. The SM difference between C_{max} and AUC is a tell-tale measure of profile discrepancy between species.

When the peak:trough concentration ratio is higher in animals than in humans, the risk level for humans will be lower when the nature of the dose-limiting toxicity suggests that the toxicity is probably caused by a transient high peak concentration that is observed in animals but unlikely to occur in humans, rather than if it is caused by sustained exposure over time. Judgement for this differentiation will be very difficult because the mechanism of toxicity, especially that of an off-target issue, is usually poorly understood and any theory will be difficult to prove experimentally. Furthermore, sustained damage might have been caused over time by seemingly transient and reversible observations and damage observed in one system could have been secondary to that of another.

The common practice of infrequent dosing in animal experiments suggests that dosing frequency could be a blind spot in translatability discussion. Better animal□human experimental translatability requires closer collaboration, through deeper engagement and mutual education, among drug developers in biology, toxicology, pharmacokinetics and drug disposition, clinical pharmacology and formulation. Regulatory agencies can also play an important part. Protecting subjects in clinical trials and ensuring safety and efficacy of approved medicines should be the primary role of regulators. However, ultimately, a government agency is for protecting and enhancing the wellbeing of citizens. In healthcare, the wellbeing largely depends on the availability of effective disease treatments. Although regulators do not have the responsibility to regulate animal study design, they can have the scientific strength and broad experience that many sponsors lack, to provide valuable guidance and advice on principles of animal study design for greater human relevance.

Enhanced dosing practice

Dose splitting is a well-established strategy in patient care for mitigating toxicity while maintaining efficacy. It raises trough concentration and lowers peak concentration, keeping overall exposure unchanged. The established utility of dose splitting for overcoming observable tolerability issues in clinical practice implies that the approach would be effective in principle for

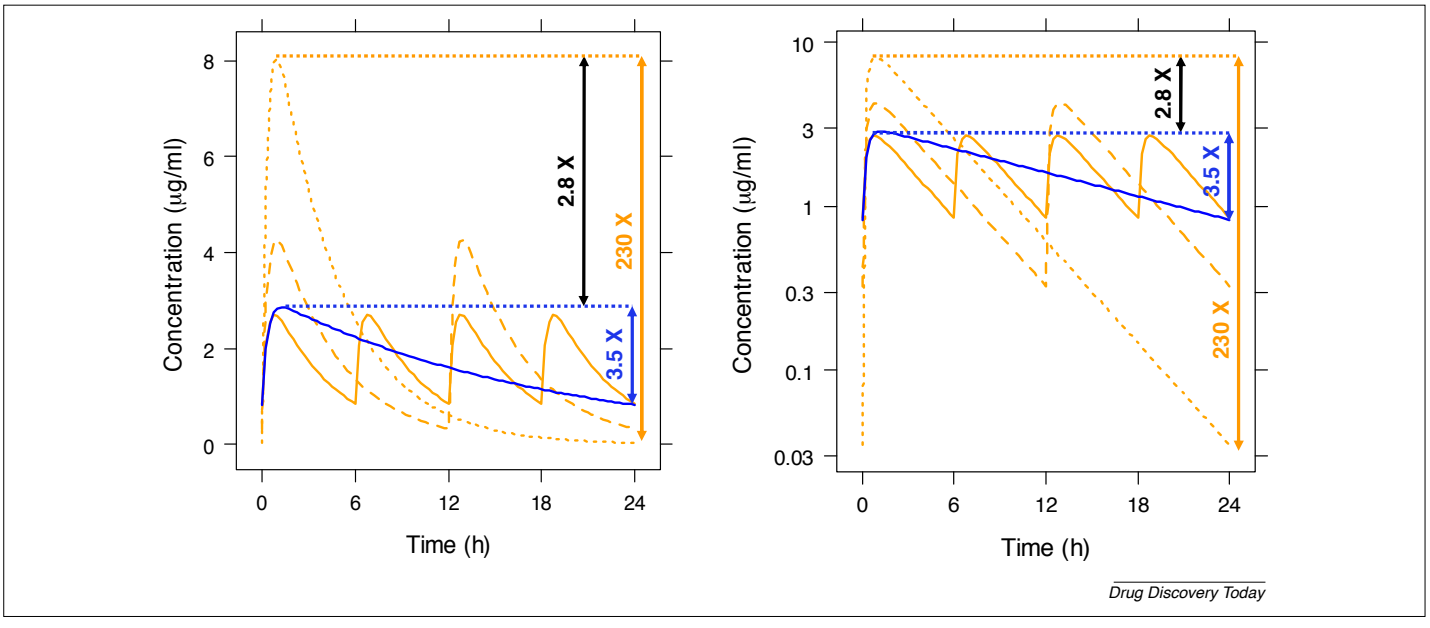

FIGURE 3

Concentration profile in animals (orange, half-life 2.9 h) receiving 10 mg/kg/day with once-, twice- or four-times-daily regimen, compared with the profile in humans (blue, half-life 12.5 h) receiving 160 mg once daily. Linear scale on the left and semi-log scale on the right. The once-daily frequency produced 3.5-fold fluctuation in humans but a 230-fold fluctuation in the animals, with higher peaks (higher toxicity risk) and lower troughs (lower efficacy potential). Despite the total exposure being the same, the peak concentration is 2.8-fold higher in the animals.

improving TI, increasing NOAEL and broadening SM, whether the toxicity is readily observable or not. Most immediate-release chemical medicines approved for human use are dosed more than once-daily. A drug□s half-life is usually shorter in animals; hence, it is only natural to dose them more frequently.

Conceivably, designing an animal dosing regimen to mimic the anticipated human exposure profile should generally improve the cross-species translatability. This is possible because the half-life in animal species is usually known at the lead optimisation stage. However, matching the animal exposure profile to the anticipated human profile is as challenging as it is important. Frequent dosing to animals is labour intensive; the logistic demand is associated with extra cost; frequent invasive dosing such as oral gavage to small animals is unfavourable for ethical and safety reasons; and extensive handling of some animals can create tolerability issues. Yet, some alternatives to overcome these obstacles have been successfully deployed: dietary dosing, subcutaneous drug depot, infusion pump, early development of prototype sustained-release formulations and a combination of systemic and local dosing to assess safety and tolerability of systemic and local exposure.

These approaches usually increase development cost and time, and they can have their own unique challenges. For example: dietary dosing is associated with circadian variation, inaccurate dose estimation and variable concentrations; subcutaneous depot can cause reactions irrelevant to the intended clinical dosing route, yet the irrelevance could be impossible to ascertain especially if the reaction is manifested systemically (such as an inflammatory response); and infusion pumps can cause reactions specific to the unnatural experimental setting. Many compounds do not have the required physical, chemical or biological properties for the alternative formulations or dosing routes. The challenges should be recognised as certainly not trivial; but project teams must think hard and be creative. Giving in to them means accepting increased risk of giving up a potentially effective therapy, so the decision needs to be a carefully considered one. Animal experiments with limited translational, hence predictive, value for human efficacy or safety are not only difficult to justify regarding resource but also raise ethical concerns over animal use.

As shown in Fig. 2, further SM erosion occurs when infrequent administration is planned for human trials. For human studies, frequent dosing can discourage trial recruitment and compromise compliance. However, consider paracetamol, an effective analgesic and perhaps the most widely used medicine today. It is well known to be hepatotoxic in overdose. But the drug is generally considered safe, without the need for a prescription if 0.5□1.0 g is taken every 4□6 h, up to four-times and at up to 4 g total dose per 24 h [15,16]. Hence, investigators and patients who are truly in need should conceivably be motivated to participate in clinical trials for drugs with well-presented clear and convincing evidence of genuinely differentiated therapeutic benefits. And early investigation into an alternative formulation, a different administration route or even a prodrug approach could help enhance acceptance and adherence.

Concluding remarks

Despite their high potency and selectivity, a large proportion of investigational drugs fail to achieve preclinical candidate selection or progress to clinical trials thereafter. This analysis, based on pharmacological principles, aided by computer simulations and supported by broad observations across drug_discovery organisations, suggests that the termination of many drug candidates could be due to the erosion of the therapeutic index or safety margin by insufficient dosing frequency in animal experiments. Loss of potentially good drugs before human testing represents an economic cost to society, reduced productivity of the drug industry and a missed opportunity for new treatments for patients. The analysis highlights the needs of closer collaborative efforts among scientists of different disciplines, regulatory guidance and better dosing practice for animal experiments to enhance clinical translatability.

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