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# MICRODOSING: A NEW STRATEGY FOR DRUG DEVELOPMENT PROCESS

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## ABSTRACT

New drugs are a great need for clinical conditions but unfortunately development costs are rising and number of drugs receiving marketing approval has fallen. Microdosing is a new experimental approach that offers a faster and potentially less expensive approach for obtaining human *in vivo* pharmacokinetic (PK) data in early stages of drug development. The concept of microdosing involves the use of extremely low non-pharmacologically active doses of drug candidates to define their PK profile in human subjects, using highly sensitive analytical techniques such as Accelerator mass spectrometry (AMS), Positron emission tomography (PET) and Liquid chromatography-mass spectrometry-mass spectrometry (LC/MS/MS). In this review we have discussed various aspects of microdosing such as regulatory requirements, methodology, validation, experimental proofs and future aspects. In conclusion, progress on three fronts, namely analytical, regulatory and understanding the role of PK in drug development has bought pharmaceutical industry to a position where microdosing can be considered as a possible first step in clinical investigations and eventually all first in human studies will commence with a phase 0 study.

**INTRODUCTION TO THE CURRENT DRUG DEVELOPMENT PROCESS:** Drug development involves basically 3 major stages: Discovery, Preclinical Investigation and Clinical Development. In each stage, the candidate compound (New Medical Entity) has to prove itself on number of fronts, i.e. pharmacodynamic, pharmacokinetic and financial aspect. Drug development process involves lots of money (around $ 600 million and above) and time (around 12 years).

**Problems with Current Drug Development:** New drugs are great need for clinical condition but unfortunately development costs are rising and number of drugs receiving marketing approval has fallen [2]. Pharmaceutical Research and Development (R&D) is a complex business. Its management requires clear strategies and decision making processes to tackle the trade-offs in cost, time, product value and success probability that occur within individual projects and across product portfolios. The importance of this endeavour is underlined by a stark reality that 75% of the cost of drug development is on failures concentrated in early stages [3] and reducing the cost of this failure- either by falling the candidates sooner or by improving the overall probability of success is the most powerful solution for improving R & D productivity [4, 5].

Out of the compounds entering clinical studies it is estimated that only about one out of 10-20, depending on the indication and class of compound, will progress

to marketing approval. Frequently these candidate drugs reach a relatively late stage of development before being discarded resulting in unsustainable losses of time and money. This late-stage attrition can kill projects, companies, jobs and sadly, probably also patients.

**Root- cause analysis of the Attrition problem:** In **Fig. 1** Root-cause analysis of the 'attrition problem', which is done by using Fish-Bone analysis (FBA) methodology, several factors are enlisted which contribute to the problem. As mentioned earlier this attrition problem leads to major loss of money and time of both, industry as well the patients.

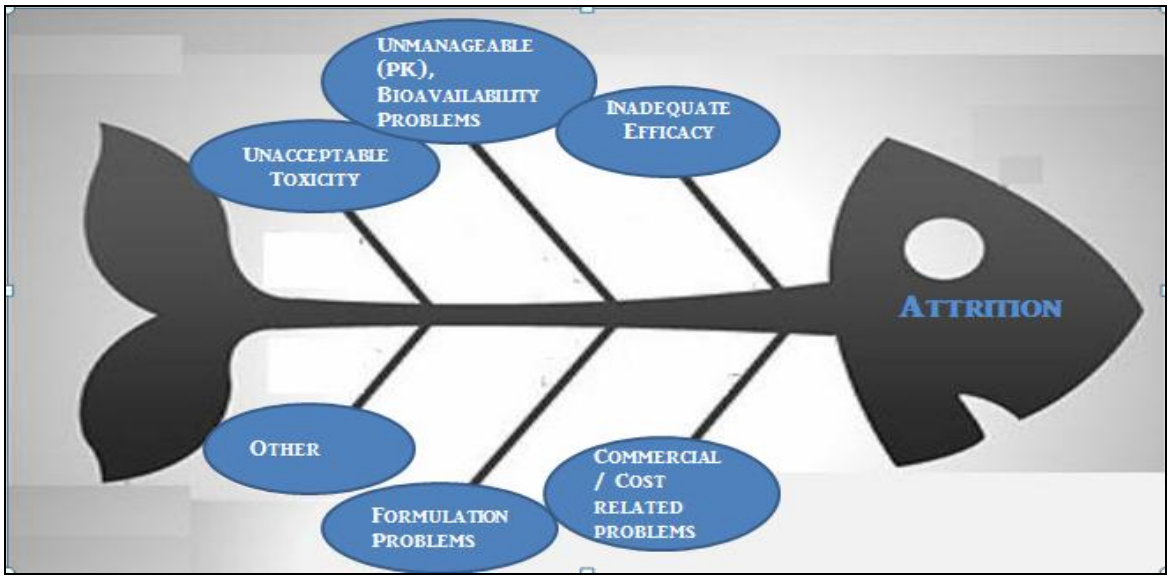


**FIG. 1: ROOT-CAUSE ANALYSIS OF THE ATTRITION PROBLEM**

According to SPS i.e. Structured Problem Solving, there is a need to target the Root-Cause which contributes more towards the problem and by targeting which we can gain substantial success in achieving our final goal i.e. to reduce the loss of time and money. After analyzing each cause, unmanageable Pharmacokinetic (PK) seems to be a main culprit. Retrospective analysis has revealed that drugs fail for various reasons such as: inadequate efficacy, unacceptable toxicity, unmanageable pharmacokinetic properties or a combination of these.

We are reliant on information gained in preclinical studies to predict PK parameters. One of the major difficulties with animal Absorption, Distribution, Metabolism and Excretion (ADME) studies is extrapolating the results to humans. Species differences in route of metabolism can cause problems and the scaling process may involve consideration of species weight, lifespan, metabolic rate, rate of cell division and body surface area [6]. Differing approaches can yield results varying by several orders of magnitude, with each step in the scaling process adding to the degree of uncertainty in the final extrapolation.

Ethical aspect involved in animal studies is also a major area of concern.

- No of animals used in 2001-2002 for R & D (safety + ADME+ Efficacy) in Britain is 5, 32, and 204.
- Observation study of 150 compounds revealed that the data from Rodent + non Rodent true positive concordance rate was 71%.
- Rodent data alone - 43% predictive.
- Non- Rodent data - 63% predictive.

As seen from the above numbers, testing the safety and efficacy of a successful human medicine can involve thousands of laboratory animals, sometimes causing considerable suffering and distress. UK [7] and European legislation requires the Replacement, Reduction and Refinement of animal procedures (the

Three R's) wherever possible, and this legislation applies fully to the development and assessment of novel medicines. The importance of the 'Three R's' concept was emphasized in a position paper adopted by the Committee for Proprietary Medicinal Products [8] of the European Agency for the Evaluation of Medicinal Products in 1997 [9].

The pharmaceutical industry thus has the dual responsibility to produce safe and effective medicines while implementing the 'Three R's' at every stage of drug discovery and development. In this respect, there has been substantial progress with applying *in vitro* and *in silico* methods to both efficacy and safety testing. In contrast, there has been little discussion about the role that human volunteer studies might have in the very early stages of drug development to replace or minimize animal experiments, and to enhance the scientific relevance of the data obtained. Thus, solving Pharmacokinetic - predictability problem can aid in minimizing the animal use in rational and scientific manner [10].

Unmanageable PK also contributes significantly in following issues mentioned in FBA:

- Toxicity: While wrong concentration reaching wrong target for longer time or metabolism issues.
- Inadequate Efficacy: Very rapid elimination or too low concentration of drug at the target organ for lesser time.
- Cost Problems: for some drugs which are expensive to manufacture, PK properties may be found such that the drug becomes uneconomical to produce [2].

**Corrective And Preventive Action (CAPA):** Therefore, all leading regulatory authorities started finding Corrective And Preventive action (CAPA) for 'Unmanageable PK issue' and the answer was to have some technique that would aid in differentiation of promising candidates from those who do not hold future development. 'Early Clinical Studies' were looked forward as a promising answer. Thus for the purpose of reforming the strategy of early clinical studies European Medical Evaluations Agency (EMEA), United States Food and Drug administration (USFDA), Japanese Society for the Study of Xenobiotics (JSSX) start thinking and evaluating newer concepts that could help [10].

- The European regulators (EMEA) published a position paper in 2003 [11]. In the paper, the EMEA approved a single dose study using a microdose techniques with a dose less than 1/100th of the dose calculated to yield a pharmacological effect and/or a maximum dose of 100 µg, under the condition of the minimal preclinical safety package.
- Following this announcement of microdose concept by the EMEA, in March 2004, USFDA in their "Critical Path" report expressed the need for a tool which enables differentiation of those candidates which hold promise from those who do not; in early stages.
- To reduce time and resources during early drug development on candidates that are unlikely to succeed, FDA published a new draft of guidance regulating the early human screening studies in 2004 and in 2006 new industry guidelines for early exploratory drug studies in humans.
- In Japan, Japanese Society for the Study of Xenobiotics (JSSX) had discussed the advantages of exploratory clinical study including microdose trials conducted in Japan. After devoted and scientific discussions by the members of JSSX and the government, a new organization was established in 2005 for acceleration of exploratory clinical studies in Japan.
- Under the current global stream of exploratory clinical studies, international discussion of ICH-M3 started in late 2006 to harmonize essential preclinical programs for the study.

And Thus 'Microdosing' came into the picture, which is also termed as 'Phase 0' (as it is done before Phase I trial) and 'Exploratory Investigational New Drug Application - ExpIND or eIND' [10].

**Microdosing: a screening test for New Medical Entities (NMES):** Microdosing will act as a screening test (**Fig. 2**) for drug candidates (NMEs) and allow only pharmacokinetically promising compounds to go further in clinical trial and thus will have significant role in reducing animal use. And thus, time, money and efforts that would have been wasted in further development of compounds with suboptimal PK can be utilized in constructive manner. The fundamental concept of Microdosing is that under conditions of pharmacokinetic dose proportionality, pharmaco-kinetic data obtained at lower doses can be used to predict PK at higher doses [11].

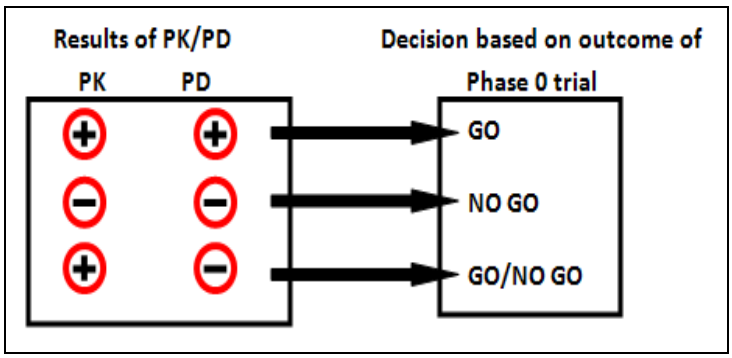


**FIG. 2: MICRODOSING - A SCREENING TEST (GO/NO- GO ASSESSMENT)** [12]

So, a microdose is a so small dose, which it is not intended to produce any pharmacological effect when administered to humans and therefore is also unlikely to cause an adverse reaction[13].

1. **EMEA and FDA Definition of Microdose:** "In the current context, the term 'Microdose' is defined as less than 1/100$^{th}$ of the dose calculated to yield a pharmacological effect of the test substance based on primary pharmacokinetic data obtained *in vitro* and *in vivo* (Typically doses in, or below, the low microgram range) and at a maximum dose of less than or equal to 100 microgram [11, 13]."

2. **Prerequisites and Preparation for a Human Microdosing Study:** The documents produced by the European and US regulatory authorities that address human Microdosing give guidance on prerequisites [11].

**2.1. Nonclinical Studies to Support Single Microdose Studies in Humans**: The toxicology evaluation recommended for an exploratory IND application is more limited than for a traditional IND application[14] as illustrated in **table 1**.

**TABLE 1: REDUCED NON-CLINICAL PROGRAMS**[15]

| EMEA Position Paper | FDA Exploratory IND |
|---|---|
| • Single dose toxicity study in one species at one dose-level with 1000x safety factor relative to human microdose; | • Single dose toxicity study in one species at one dose-level with 100x safety factor relative to human microdose; |
| • 14 days extended observation with interim sacrifice on day 2; | • 14 days extended observation with interim sacrifice on day 2; |
| • Intended human route of administration and i.v. Route | • Intended human route of administration only; |
| • Genotoxity according to ich m3 or abridged | • No genotoxity studies required |
| • Comparative *in vitro* metabolism and pharmacodynamic data | • Comparative *in vitro* metabolism and pharmaco- dynamic data |
| Total time for program: 10-12 weeks from receipt test substance/documentation to draft reports Test substance required per compound : ~7 gram | |

Logically the authorities note that any observed toxicity may require further clarification and that all nonclinical studies should be conducted according to Good Laboratory Practices (GLP) [11].

**2.2. Design of Phase 0 Clinical Trial**: Microdosing is a new experimental approach (**Fig. 3)** which believes in the principle that 'the best models for humans are humans', hence sub-pharmacological doses of drug are administered to human subjects to provide early human data on basic PK parameters, such as clearance, volume of distribution and half-life [16].

Before Human Microdosing (HMD), researchers might typically identify one or more drug candidates that have demonstrated pharmacological activity *in vitro* and in animal models. After administration of doses (oral and/or intravenous) to human volunteers, relevant body-fluid samples are collected for subsequent analysis.

The ADME data from microdose studies can be fed into in silico PK models to obtain a much better estimate of the probable pharmacological dose in future efficacy studies [5].

This parallel way of conducting microdose studies is most appropriate when several drug candidates are available. Each molecule might be administered in a crossover design, such as an intravenous dose followed, after a suitable washout period, with an oral dose.

Thus, volume of distribution ($V_d$) and clearance ($C_l$) can be obtained, as well as other standard PK parameters. Information on parent drug and metabolite(s) can be obtained through chromatographic separation of an appropriate extract (e.g. plasma), followed by Accelerator mass spectrometry (AMS) analysis of collected chromatography fractions. In some cases, the drug discovery process might only yield a single molecule in those cases microdosing can be used to find correlation between animal and human effects.

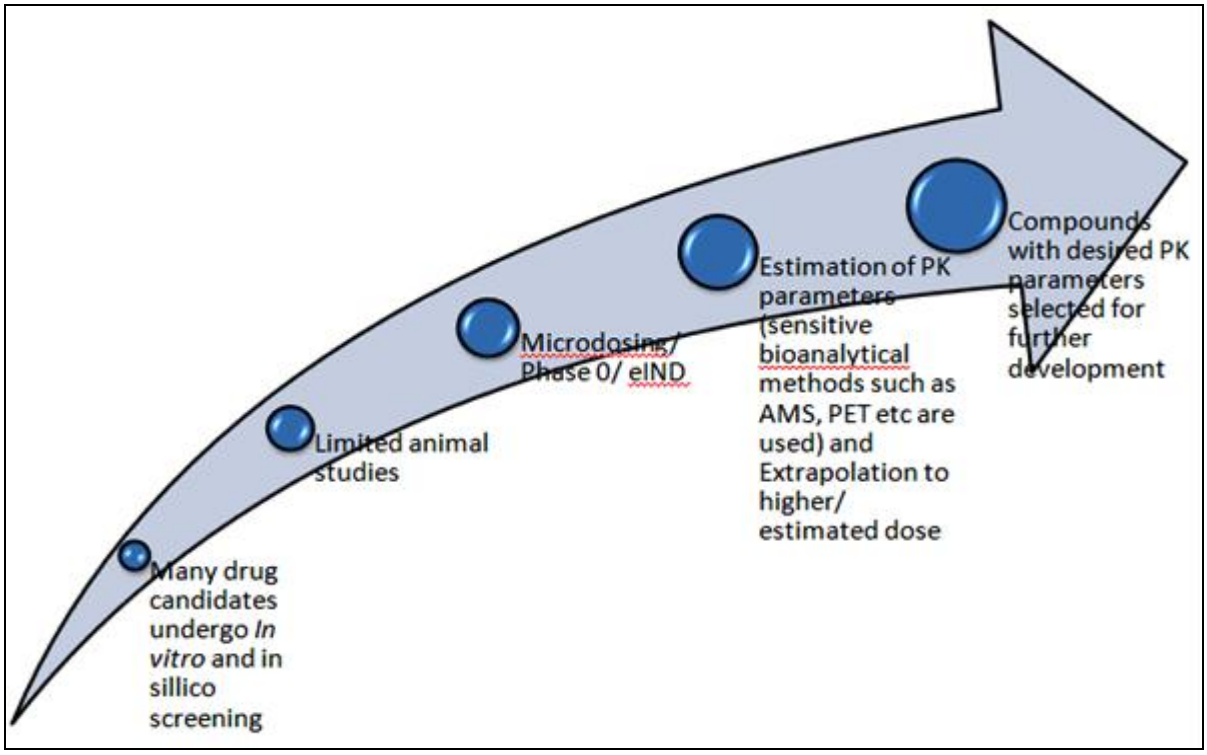


**FIG. 3: MICRODOSE APPROACH TO DRUG CANDIDATE SELECTION**

**Bioanalytical methods and the role of different Spectrometric and Imaging methods:** Because of the low drug concentrations associated with microdosing, highly sensitive bioanalytical methods are required for the accurate determination of PK parameters. It is therefore prudent to estimate the likely critical concentrations and ensure an adequate lower limit of quantification for accurate measurement. Concentration estimates can be made from a PK model which has been predicated from available preclinical *in vitro* and *in vivo* data, the intended dose and sampling times.

In addition, given the vagaries of extrapolating human models from preclinical data and the variability between human subjects, it is advisable to set the target lower limit of quantification (LLOQ) two- to threefold below the lowest estimated concentration to be measured for obtaining reasonable PK estimates [11].

Many technologies might potentially be used in very early human studies, including functional magnetic resonance imaging, single photon-emission computed tomography, chemical reaction interface-mass spectrometry and liquid chromatography-mass spectroscopy [10]. Various aspects of AMS, NMR spectroscopy and PET imaging are compared in **table 2**.

**TABLE 2: AMS, NMR SPECTROSCOPY AND PET IMAGING IN EARLY MICRODOSE STUDIES IN HUMANS**

| Method | Sensitivity | Uses | Advantages | Limitations |
|---|---|---|---|---|
| AMS | Attomole ($10^{-18}$ M) sensitivity | ADME and detailed PK with radioisotopes used with standard separation methods. | Extreme sensitivity means very low radiological and chemical hazard. | Expensive equipment (around $1 million), Assay costs approx. £200 per sample. |
| NMR spectroscopy | 4-20 µmol for 400 MW drug, depending on field strength of instrument. Improved 3-4 fold by cryoprobe technology | Drug metabolite profiles, PK, metabonomics (changes in endogenous metabolites), Monitoring efficacy or toxic biomarkers, can be coupled with standard separation methods if necessary. | Very high resolution, rapid analysis, does not require radioactive isotopes, no need to preselect molecule of interest, none or minimally invasive. | Expensive equipment, moderate sensitivity if 'stand-alone' |
| PET imaging | Picomole ($10^{-12}$M) sensitivity, high specificity, 2-4mm spatial resolution | ADME and detailed PK plus some PD using radioisotopes followed by non invasive scanning. | Permits visualization of drug disposition in target organs, very sensitive | Expensive equipment, uses radioisotopes, short life of radioisotopes imposes time constraints. |

**1. Accelerator Mass Spectrometry (AMS):** When there is a risk that adequate estimates of PK parameters will not be obtained because target LLOQ cannot be achieved using non-radiolabeled analytical methods, AMS offers an alternative solution [11]. AMS has recently been adapted for use in the laboratory for detecting extremely low levels of substances such as drugs and their metabolites, macromolecular adducts or receptor/ligand interactions, in blood, urine and faeces.

AMS separates atoms on the basis of their mass, charge and energy differences, and can individually quantify isotopes such as $^{12}C$, $^{13}C$, $^{14}C$, $^{3}H$, and $^{36}Cl$ [10]. Phase I studies are commonly conducted using $^{14}C$-labelled drugs, but this isotope has a slow rate of radioactive decay and its use normally requires quite large doses of radioactive drug to be administered.

AMS is several orders of magnitude more sensitive than liquid scintillation counting, being able to detect radiolabeled drugs at amol ($10^{-18}$ moles) levels, and with great precision. This reduces the total radiation exposure of volunteers, as well as minimizing any chemical hazards [17].

AMS can quantify levels of drugs and their metabolites, assess bioavailability and measure plasma clearance, at doses considerably below the pharmacological level. Comparisons of AMS and liquid scintillation results showed good correlation in an excretion balance and pharmacokinetics study of fluticasone propionate, demonstrating the relevance and sensitivity of the newer method [1].

Limitations of AMS: Although AMS is in a league of its own in terms of analytical sensitivity it does require synthesis of a radiolabeled version of the test compound which can in some cases be problematic. This may have time and cost implications. Analytical costs using AMS are also high because of the highly sophisticated equipment involved and the need for sample fractionation to measure parent compound and individual metabolites [11].

However, the availability of AMS and its ease of use have improved considerably during the last decade and consequently the expenditure associated with the technique has dropped substantially, reducing the cost of analyzing a sample significantly. This is partly as a result of the technology having become more mature and therefore more reliable and partly due to the fact that new compact and less expensive accelerators have been introduced into the market. This has facilitated successful commercialization of AMS into the pharmaceutical and biochemical field [19].

**2. Nuclear Magnetic Resonance (NMR) Spectroscopy:** NMR spectroscopy measures the presence of atoms such as $^{1}H$, $^{13}C$, $^{19}F$, which are non-radioactive stable isotopes and $^{31}P$. Coupled with an analytical system, such as high-pressure liquid chromatography or high-pressure liquid chromatography–mass spectrometry, NMR spectroscopy can determine drug metabolite profiles, the pharmacokinetics of multiple metabolites in one study, and can be used in metabonomics [20, 21].

NMR spectroscopy can be applied to the analysis of body fluids, such as urine, saliva, plasma, blood cells, biopsy material, cell aspirates. The technique provides a 'fingerprint' of biochemical changes characteristic of the nature or site of a toxic (or other) effect, so there is no need to pre-select molecules of interest. Metabonomics can be used to develop and monitor biomarkers of efficacy or toxicity and to identify sites and mechanisms of toxicity. For example, NMR spectroscopy of urine, plasma and tissues has been used to identify biomarkers of altered energy metabolism, specific target organ damage, altered gut microflora metabolism, metabolite profiles of disease models and many other pathological or physiological changes.

With regard to concerns about whether NMR is an appropriate technique to use for the above purposes [22], have shown that cryogenic probe technology can significantly compensate for the inherently low sensitivity of natural abundance of $^{13}C$ NMR spectroscopy. This observation permits the routine use of NMR spectroscopy of body fluids, with acquisition times conducive to high throughput screening [10].

**3. Positron Emission Tomography (PET):** PET is a sensitive technique in which a compound labelled with a short-lived, positron-emitting isotope is administered to a volunteer, followed by non-invasive scanning. This yields three-dimensional quantitative pharmacokinetic (and pharmacodynamic) data for a candidate drug in human volunteers. With PET, doses as low as 0.1–1.0% of the Phase I starting dose are used in pharmacokinetic studies to provide ADME data, including hepatobiliary and renal clearance [23].

PET has been used to acquire pharmacodynamic data, such as drug-receptor interactions, quite extensively in neurology and psychiatry [24] and now increasingly in oncology [23]. Regional kinetics can be used to obtain values for receptor numbers, affinity and binding potential. Drug receptor studies are useful for assaying targets and predicting responses to new drugs, and for assessing optimal doses. PET can monitor other targets too, including genes, enzymes, neurotransmitter transporters and pathways involved in signal transduction.

An example of the use of PET in the above way is a pre-Phase I trial of *N*-[2(dimethylamino)ethyl]acridine-4-carboxamide (DACA) in cancer patients, to evaluate the pharmacokinetics of the drug [25]. The study measured the distribution of the drug and its metabolites in a variety of tumours and normal tissues. The plasma metabolite profile of the drug, its plasma clearance and the maximum radioisotope concentrations in the brain and vertebrae compared with other tissues were also measured.

Anti-cancer drug development is undergoing rapid changes, and early proof of principle relating to mechanism of action is being demonstrated in Phase I and pre-Phase I human studies, often using PET. Similar PET studies are also feasible in other fields of drug development [26].

**4. Liquid Chromatography-Mass Spectrometry-Mass Spectrometry (LC/MS/MS):** In techniques such as AMS and PET the dosing of 14C-labeled drugs, or "hot drugs", is absolutely essential and the synthesis involved are both costly and time consuming. It puts financial burden on the pharmaceutical company and can cause temporary suspension at development stages. Additionally, the process for sample treatment takes extra man-hours. In contrast to this is LC/electrospray ionization (ESI)-MS/MS, which has been used most frequently in the analysis of drugs in human matrices. ESI makes it possible to ionize almost all polar compounds [27]. The most remarkable aspect is that determination of concentration in plasma can be done after administration of a non-labelled drug (cold drug).

Many reports on actual examples of determination of drugs at a picogram per millilitre level in human plasma using LC/ESIMS/MS [2]. Studies conducted to evaluate the accuracy and usefulness of LC/ESIMS/MS concluded that the quantitative determination of drug at a picogram per millilitre order in human plasma using LC/ESIMS/MS was useful and effective for a microdosing study.

There is the potential that analysis using LC/ESI-MS/MS could be advanced to being utilized for a microdose clinical trial with a cold drug (non-radioisotope-labelled drug) [29]. Some cross comparison studies have been carried to compare and correlate the results from different methods, e.g. AMS and LC/MS/MS, PET and AMS and results were found to be comparative [30, 31].

**Validation of Microdosing Strategy:** An aspect of microdosing is the evaluation of linearity of pharmacokinetics between the microdoses and the therapeutically equivalent doses of new chemical entities, leading to predictability of pharmacokinetic parameters at high doses from the microdose data.

Before this aspect is applied in the clinical setting, it is prudent to demonstrate linearity in pharmacokinetic parameters in relevant human as well preclinical models that shows pharmacokinetic properties and metabolism broadly similar to those projected in humans [32].

Two representative examples of these types of studies are as follows:

**1. The CREAM trial: a tough test of the Microdosing Concept:** Nonlinearities in PK parameters could be induced when binding, metabolizing or eliminating systems become saturated. To address the issue of non-linearity, a collaborative industry sponsored trial [Consortium for Resourcing and Evaluating AMS Microdosing (CREAM)] was undertaken, which included several drugs for which it was difficult to predict human PK because of, for example, high first-pass effects. Each of the compounds was administered to subjects at a microdose level and at a therapeutic dose level in an appropriate randomized crossover design.

The trial was set up to be a rigorous test using compounds that were expected to pose a considerable challenge to the microdosing concept. Of the five drugs investigated, microdose PK data reflected pharmacological dose PK for three compounds, and gave important metabolism data for one drug (unfortunately one compound was a no-test). Although this study was not exhaustive, it demonstrated ~70% correspondence between microdose and pharmacological dose PK. Considering that the compounds used in the CREAM trial were selected because of their challenging PK properties, the CREAM trial to be a considerable success [16]. Details of the study are given in **table 3**.

**TABLE 3: DETAILS OF CREAM TRIAL**

| Sr. No. | CREAM trial drug | Selection rationale | Microdose result |
|---|---|---|---|
| 1 | Warfarin | Stable *in vitro* but exhibits extensive, albeit slow metabolism *in vivo* substrate for CYP2C9 | Not predictive: although slow metabolism and long half life identified. |
| 2 | Midazolam | A selective substrate for CYP3A4<br>High first pass metabolism | Predictive: excellent correlation of key PK parameters |
| 3 | Diazepam | Low clearance, basic compound eliminated via CYP2C19, Linear kinetics over a range of doses (possibly not at microdose | Predictive: excellent correlation of key PK parameters |
| 4 | Erythromycin | Substrate for CYP3A4 and the intestinal efflux transporter P-glycoprotein | Issue in administration: no test |
| 5 | ZK253 (drug candidate dropped after phase I) | Bioavailability difficult to predict from animal models, Low bioavailability in humans | Predictive: extremely low bioavailability was identified |

Abbreviation: CYP- cytochrome P450

**2. Validation of an analytical method and checking suitability of the compound for Microdosing Study:** Preclinical species that can be used for allometric scaling to project human pharmacokinetic parameters may be used as a preclinical model for microdosing. More than one preclinical species could also be used to strengthen the projection of linearity in humans, thereby increasing confidence in the utility of microdosing studies in humans [33]. Detailed account of one such study is given in **table 4** and **Fig. 4** along with observations and conclusions of the experiments.

**TABLE 4: DETAILS OF ONE OF SUCH STUDY [33]**

| Drugs | Fluconazole | Tolbutamide |
|---|---|---|
| **Animal Species** | Rats | Rats |
| **Route of administration** | Oral | Oral |
| **Analytical Method** | LC/MS/MS | LC/MS/MS |
| **Doses** | 0.001(●), 0.005(○), 0.05 (▼) and 5(∇) mg/kg | 0.001(●), 0.002(○), 0.01(▼), 0.1(∇) and 1(■) mg/kg |

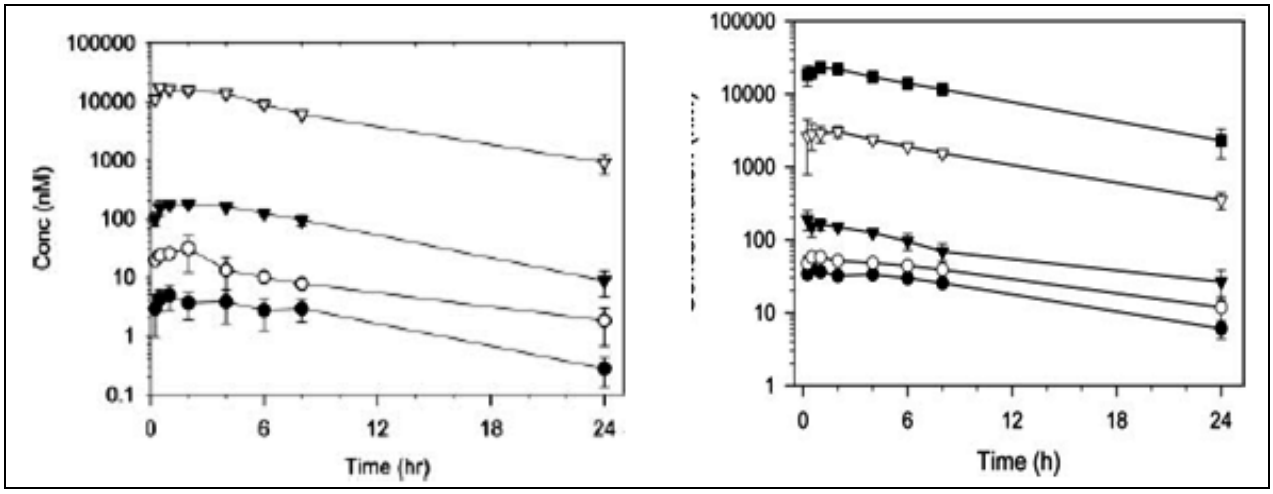


**FIG. 4: PLASMA CONCENTRATION-TIME PROFILES OF FLUCONAZOLE AND TOLBUTAMIDE RESPECTIVELY**

**Observations:** Both the drugs included in this study are reported to show linear increase in exposure after oral administration in humans [32, 34] and also have similar metabolism in humans and rats [35, 36, 37, 38].

**Conclusion of the study:**

1) Graphs in **Fig. 4** give the assurance that linearity assumption is valid.

2) This assay can further be used for the validation of different bioanalytical methods.

3) This assay procedure can be used for NMEs with unknown PK profile, and thus to make decision: whether or not the microdosing study can be conducted to give predictable data or the traditional (preclinical to phase I directly) approach to be followed.

Such type of studies will not only help in validating and exploring different aspects of microdosing but also in developing a solid database for microdosing [34].

**Applicability and advantages of Microdosing:** Progress on three fronts, namely, analytical, regulatory and understanding of the role of PK in drug development, has bought pharmaceutical industry to a position where a microdosing study can be considered as a possible first step in clinical investigations [11].

Applications of microdosing are summarized in **Fig. 5** whereas in **table 5** comparison of microdosing strategy and conventional Phase I approach.

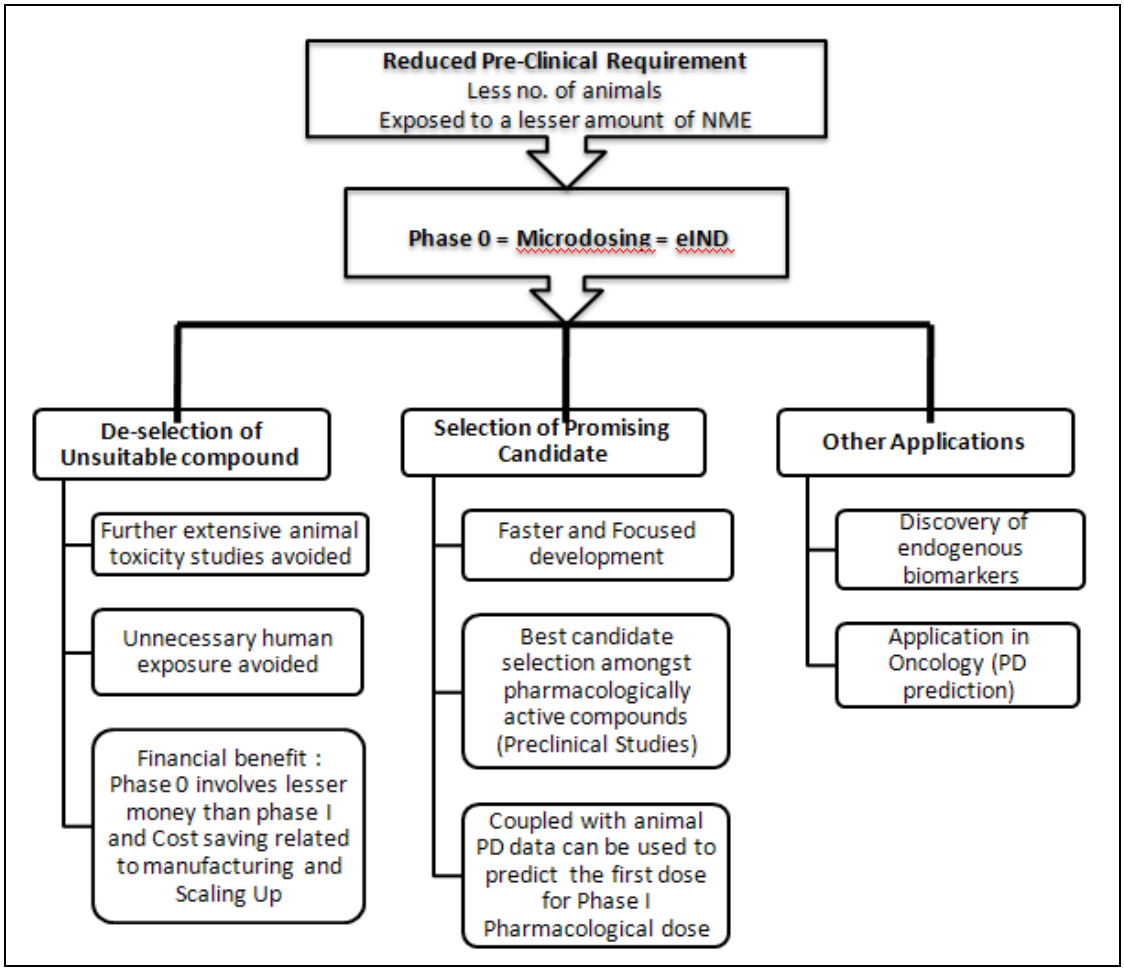


**FIG. 5: APPLICATIONS OF MICRODOSING**

**1. Impact on animal use:** The microdose is so low, and therefore the risk of an adverse event too small, that the animal testing required before administering the compound to humans can be substantially reduced. The regulatory requirement is that testing need only be conducted with single doses in one animal species, usually rat. Therefore, animals are still necessary to establish the microdose in humans, but at a much reduced level.

Also, microdosing in humans has the potential to replace animal use in determining suitable pharmacokinetic profiles of compounds. If microdosing in humans turns out to be more predictive than the current animal methods, there would be less drug candidates proceeding through extensive safety and toxicology testing involving animals, because more compounds will be terminated after early microdose studies.

**2. Impact on financial aspect:** The cost of conducting a microdose study is phenomenally less, as compared to a full phase I study. A conventional phase I study may cost about US $1.5- 5 million, whereas in the microdosing approach, the cost drops to about US $ 0.3-0.5million [39]. The dose of a compound required to conduct toxicity studies will probably be significantly lower than when using an MTD approach and the amount can be predicated accurately in advance.

With a Maximum Tolerated Dose (MTD) approach, it is impossible to predict the amount of compound which will be required until the MTD is actually established. This situation creates problems for synthetic chemists producing candidate compounds and often leads to over estimating drug requirements, as well as long delays in initiating the clinical study [10].

During drug development, when a large number of me-too compounds are screened and found to have similar or differing animal pharmacokinetics, comparative human microdose studies can be done to establish pharmacokinetics [40]. This pharmacokinetic data can further be used to help in selection of the ideal candidate drug [41], establish the tentative pharmacological dose [42] and calculate the probable cost of the deliverable.

**TABLE 5: COMPARISON OF MICRODOSING STRATEGY AND CONVENTIONAL PHASE I APPROACH**

| Features | Microdosing strategy | Conventional Approach |
|---|---|---|
| Time from preclinical to first in man studies | 6-8 months | 12-18 months |
| Cost of early phase of drug development | US $ 0.3-0.5 million | US $ 1.5-5.0 million |
| Amount of drug required | < 100 micrograms | About 100 gm |
| Special requirements | $^{14}C$ labelled Compound, if using AMS | None required |
| Regulatory requirements | Very few and limited GLP to be followed for synthesis | Established firmly GMP to be followed for synthesis |

**3. Impact on Human Exposure:** Microdosing approach works on the principle that "Humans are the best models for Humans". Thus, the problem of unpredictable PK can be solved with this approach. As mentioned earlier, preclinical data is less predictive because of the species difference, unavailability of suitable animal models, metabolic differences in animal species and human body as well, the preclinical data cannot predict the racial or individual differences in drug PK.

All these questions can be answered by Phase 0. A microdose is so small that when administered to human subject, it is not intended to produce any pharmacologic action hence; the risk of adverse events is less rather none. Combined with *in vitro* and *in vivo* metabolism comparisons between animals and human, the human microdosing data could then be utilized to predict human pharmacokinetics at the therapeutic dose [10, 42].

**4. Discovery of Endogenous Biomarker:** Human microdosing promises to be a significant analytical tool. Microdosing could be useful in the discovery of endogenous biomarkers, which would assist in the quantitative evaluation of the *in vivo* effects of drugs [10].

**5. Application in Oncology:** In oncology phase 0 clinical trials are welcome in a big way [43]. The mechanism of clinically testing new oncology drugs has not changed substantially in 40 years. The failure rate for new oncology drugs is currently over 90% [44]. Phase 0 trials provide an alternative early drug development paradigm that addresses some of the current pitfalls. This new paradigm incorporates validated assays for assessing PD and PK early in clinical development to potentially expedite rational drug development.

These studies are designed with the objective to establish the very earliest opportunity before a large number of patients have been accrued and exposed to potential drug-associated toxicity-whether an agent is modulating its target in a tumour, and consequently whether further clinical development is warranted [43]. Through this mechanism, drugs unlikely to have a therapeutic effect may be deprioritised early in the interest of furthering the expeditious development of more promising and potentially efficacious agents [44].

**Areas of concern regarding Microdosing:**

**1. Radiolabeled Compound Administration:** Radiation dosimetry, relevant to AMS and PET, but not to NMR spectroscopy, is a major safety concern. Different countries vary in their requirements on the limits for exposing volunteers to radioactive substances. The World Health Organisation restricts radiation exposure in volunteers to 1 millisievert (mSv), but more commonly 0.5 mSv is used. The International Commission on Radiological Protection guidelines[45] state that exposure below 0.1 mSv does not require regulatory approval. Human ADME studies with AMS can be conducted at exposure levels of approximately

0.9 µSv of labelled drugs [46], and so they are well below this level and also the level requiring specific approval from the UK Department of Health's Administration of Radioactive Substances Advisory Committee. This could save development time and resources. Many PET ADME studies can be conducted at radiation exposures below 1-2 mSv, it is much less than what is regarded as an acceptable level of genotoxic impurity. For microdose studies conducted in Britain by using a $^{14}C$-labelled drug, minimal animal dosimetry studies are required to predict the radioactive exposure. However, in other countries such as Netherlands, animal dosimetry is not required for very low-dose exposure to radiolabeled drugs [10].

**2. Concerns regarding the Linearity Assumption:** Concerns about saturable metabolism and transport are often raised when the use of microdosing is looked forward. It should be acknowledged that, due to these possibilities of non-linearity, some further research on extrapolating from microdoses to therapeutic dose-levels is required [10]. As mentioned in the validation part, methods for checking compound's suitability for microdosing can be developed.

**3. Concerns regarding no therapeutic benefits to the patients:** As the dose of the compound involved in microdosing is very small, there is no therapeutic benefit to the patients (specifically in oncology trials: no whole body effect, only cellular response). However, the patients involved in phase 0 studies can be enrolled in further or different trial or treatment. Also, as the dose is so small, the washout period is also less. The duration of the phase 0 is also small [12].

**4. Concerns regarding Solubility:** Some compounds dissolve readily at microdose yielding good absorption characteristics; however, at therapeutic doses, they exhibit limited solubility and absorption becomes dependant on the rate and extent of dissolution, which cannot be predicted at microdose level. More attention towards this is warranted [2].

**5. Requirement of expensive Analytical Tools:** The techniques required such as AMS, PET etc are very expensive and sophisticated because the concentration ranges to be estimated are very less.

**6. Limited Data Base and less awareness:** As phase 0 concept is still in its infancy, limited database [2] is available. Also more awareness needs to be spread.

**7. About the maximum limit set by Regulatory authorities:** There are concerns about the maximum limit set by Regulatory authorities (EMEA and USFDA) i.e. 100 microgram. This is because of the newer advancements that can be achieved with microdosing. For example, PET makes it possible to image the path of the NME or destination achieved by NME [PD determination in oncology (cellular responses)], also if non-radiolabeled compound is to be used [26]. USFDA has approved new approaches, for enabling flexibility in the amount of dosing. Thus, more utilization of the novel concept is possible. Following two approaches are proposed:

First Approach: It involves not more than the total dose of 100 µg. Dose can be divided among up to 5 doses in any subject. This could be useful to investigate target receptor binding or tissue distribution in a PET study in addition to assess the pharmacokinetics of the test substance with or without the use of an isotopically labeled agent.

These studies could be supported by an extended single dose toxicity studies in one species usually rodent, by the clinical route of administration together with appropriate characterization of pharmacology.

Second Approach: It involves ≤5 administrations of a maximum of 100 µg per administration (a total of 500 µg per subject). This could be useful for similar applications as for first approach but with less active PET ligands. These studies could be supported by a 7 day toxicity study in one species, usually rodent, by the clinical route of administration, together with assessment of the genotoxic potential of the unlabelled compound and appropriate characterization of pharmacology.

**CONCLUSION:** Human microdosing clearly holds significant promise as an analytical tool in the coming years. As research methods and technology involved in Phase 0 trials become more sophisticated, human microdosing may be applied to a number of drugs that could potentially be administered consecutively**.** Microdosing may later become an accepted approach in drug development, when first in human studies may begin with a Phase 0 study. However, the true utility of Phase 0 microdosing studies lies with the ability to predict under what circumstances this approach will provide data within a specified and acceptable range, as compared to the therapeutic dose data.

Capitalizing on the continuing rapid advances in drug development technology, there is no question that decreasing the time of drug development, reduces the cost phenomenally. Thus, microdosing strategy could complement the standard anima-to-human allometric scaling; redefining the present phase I study designs. This strategy may help to reduce animal testing in the identification of novel drug candidates.

Further, microdosing may help both patients and the pharma industry with earlier availability of the new test medication and reduced attrition of compounds at later stages of drug development. Microdosing allows not only selection of drug candidates more likely to be developed successfully, but also helps in determination of the first as well as pharmacological dose for subsequent human studies.

And, thus microdosing unfolds more opportunities for innovation and improvement and can be viewed as one of the milestones in the drug development process.

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