

Preclinical Toxicology for Inhaled Drugs – Practicalities and Implications

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The package of preclinical safety studies needed to support administration of inhaled drugs to humans in clinical trials is generally the same as that for other routes of administration, with development plans usually following the recommendations outlined in the relevant ICH guidelines. Where possible, repeat dose toxicology studies should employ the inhaled route of administration in order to mimic the intended clinical route of administration and to ascertain any potential for adverse effects in the respiratory tract, in addition to defining the systemic toxicity of the drug. When studying the carcinogenicity of an inhaled drug, at least one of the two non-rodent studies should utilise the inhaled route of administration, however, it is acceptable to conduct some studies, such as in vivo genetic and reproductive toxicology studies, via alternative routes (typically intravenous) when it is paramount to maximise systemic exposure.

Although inhalation toxicity studies present challenges that are unique compared to those performed by other routes of administration, many of the factors influencing toxicity such as biological (strain, age and health of animals) and environmental (temperature, housing, humidity) are universal. Dosing by inhalation is generally more complex than other routes of administration, requiring considerable preliminary work to produce suitable formulations (powdered blends, metered dose inhalers and nebulised solutions/suspensions) and reproducible test atmospheres, in addition to allowing the animals to acclimatise to the dosing apparatus. During the dosing phase of a study, chamber concentrations and particle size are regularly determined. These extra procedures ultimately make the studies more labour intensive and therefore more expensive.

Rodent and lagomorph studies usually employ snout only exposure, while non-rodent studies are generally performed using an oro-nasal mask. Administration through an oro-pharyngeal tube can also be used for non-rodents when the supply of test article is minimal and precious, a short exposure time is required or when dose limiting nasal irritancy has been encountered. In the pharmaceutical industry, whole body exposure systems are largely a thing of the past due to the large quantities of test material required and the potential for cross contamination and ingestion through grooming.

Using the dosing systems available, aerosols can be successfully generated from metered dose inhalers, dry powder blends and nebulised solutions or suspensions. The mass median aerodynamic diameter (MMAD) should be kept <5µm in order to ensure adequate exposure to the respiratory tract, although it is preferable to target 1-3µm with a geometric standard deviation (an index of dispersity) of 2-3µm. If the MMAD is >7µm it is possible that the study would be considered to be invalid, having not achieved one of the primary objectives (i.e. an adequate assessment of the effect on the respiratory tract).

Unlike most other routes of administration, the exact dose administered by inhalation (mg/kg) is not known but is estimated, taking respiratory minute volume (RMV; L/minute), airstream concentration of the test material (mg/L), duration of daily exposure (minutes), body weight (kg) and inhaled fraction (%) into account using the following formula:

$$\text{Dose} = \frac{\text{RMV} \times \text{Concentration} \times \text{Duration} \times \text{Inhaled fraction}}{\text{Body weight}}$$

Of these parameters, airstream concentration, body weight and exposure time will be known. Some laboratories have the capability to determine RMV during a study, however, more often or not it is an estimate based on a function of body weight according to the formula developed by Bide and co-workers (2000) or that recently proposed by the Association of Inhalation Toxicologists (Alexander et al, 2008). For simplicity, the inhaled fraction (that is, particles of < 7µm) is often assumed to be 100%.

Dry powder blends of drug and lactose (with or without ternary agents) are currently the preferred formulation for the development of drugs to treat pulmonary diseases. For practical (reproducibility) and scientific (avoiding excessive lung overload) reasons the maximum achievable doses are often limited so that a total particulate concentration of 2 mg/L is not exceeded. Under such circumstances, high dose levels in the region 40-50 mg/kg and 20-25 mg/kg can be achieved when using a one hour exposure and a 40% (w/w) dry powder/lactose blend in rats and dogs, respectively.

The principles involved for determining toxicokinetics during inhaled studies are the same as for any other route of administration, except that extremely low doses (<10µg/kg) may be utilised and the dose is estimated rather than known. The deliberate generation of an aerosol and the wide range of doses, combined with the very low limits of quantification (e.g. 0.1 ng/mL) required to detect the drug in biological fluids at very low doses means that cross contamination between dose groups (including controls) can be an issue. However, a variety of procedures to limit transfer of drug between groups via equipment and clothing, along with the use of individually ventilated cages, can be employed to avoid excessive contamination, and potential invalidation of a study.

As for other routes of administration, safety margins for target organ toxicity (e.g. hepatotoxicity and non-genotoxic carcinogenicity) are generally based on systemic exposures of the drug in terms of plasma area under the curve (AUC), while for acute effects (e.g. tachycardia and CNS effects) the margins are often based on maximum plasma concentrations (C_{max}). Local effects generally manifest as upper respiratory tract irritancy affecting the larynx and/or nasal cavity, and inflammatory changes in the lung, resulting from irritancy or particulate overload. In terms of upper respiratory tract changes (e.g. squamous metaplasia, erosion and ulceration), the rodent larynx is extremely sensitive to insult from inhaled particles and the changes are not usually predictive of irritancy in humans, while similar histological changes in the nasal cavity are considered irrelevant to a drug administered to humans by oro-inhalation. However, the changes in the lung, which can include macrophage accumulation, cellular infiltration, epithelial degeneration, necrosis, hyperplasia and fibrosis, are considered to be a safety concern as they are non-monitorable in humans. In calculating safety margins for lung changes the drug deposited per unit weight of lung is calculated. Generally, 7, 20 and 40% of a dose can be assumed to be deposited in the lungs of rats, dogs and humans, respectively (Snipes,1988), although the FDA are relatively conservative and often assume 100% deposition in humans. The FDA also state that safety margins should be a minimum of 10 fold for rodents and 5-6 fold for non-rodents.

Finally, there are some examples where the conditions of snout only exposure can exacerbate the potential for specific toxicities in rats. Some beta₂-adrenergic agonists (e.g. formoterol) and alternative propellants (e.g. HFC-134a) have caused testicular toxicity in rats when administered via snout only exposure, while administration via other routes (oral or intravenous) or via whole body (inhalation) exposure either avoided the toxicity or greatly increased the threshold dose required. The mechanism for these changes is unknown, although heat stress has been suggested to play a role. Regardless of the cause, the snout only conditions are irrelevant to the human situation.

Therefore, whilst general principles are the same, there are some peculiarities resulting from administration of drugs to animals via inhalation. These should be considered in the design of preclinical studies and interpretation of findings.

References

Alexander DJ, et al (2008). Association of Inhalation Toxicologists (AIT) Working Party Recommendation for Standard Delivered Dose Calculation and Expression in Non-Clinical Aerosol Inhalation Toxicology Studies with Pharmaceuticals. *Inhalation Toxicology*, 2008, in press.

Bide RW, et al (2000). Allometric respiration-body mass data for animals to be used for estimates of inhalation of toxicology to young adult humans. *J Applied Tox*, 20, 273-290.

Snipes MB (1988). Species comparison for pulmonary retention of inhaled particles. In: *Concepts in Inhalation Toxicology* (Ed: RO McClellan). Hemisphere Publishing Co., Washington DC. pp 193-227