APPLICATIONS OF THE INTRATRACHEAL INSTILLATION TECHNIQUE IN INHALATION TOXICOLOGY EVALUATIONS

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Abstract

Pulmonary effects of a chemical, for reasons of space, time and economics, along with non-availability of sufficient quantities and/or sample tissues, can not always be tested following inhalation exposures. Uses of intratrachael administration involves methodology that is simple and uses relatively little material so that risk to laboratorians is much reduced (from an inhalation study). One can deliver relatively large amounts of material to the lung in a short period of time.

Overriding these advantages however, is the primary fact that amounts and sizes of particulate that would otherwise not be able to gain access to the lung, do so. The patterns of particle distribution in the lung following instillation are uneven and are unlike those resulting from inhalation. The nonuniformity is partially random but represents systematic and reproducible regional differences. Another serious problem is that the instillation technique totally bypasses the upper respiratory tract. Problems can result from altering dosing rates and the use of differing suspending agents. Despite the advantages stated earlier, these latter concerns have alerted the experimental inhalation toxicologist to the limitations of results obtained using this technique.

Introduction

The potential toxic effects that need to be appreciated following inhalation exposure include irritation of the respiratory tract, behavioral changes, pathologic change to vital organs or tissues within the distal to the respiratory tract, immune system responses, pulmonary function alterations, metabolic disturbances, carcinogenicity, and even death. Studies to measure the effects of chemical and physical agents on the biological system after entering the respiratory tract must follow carefully designed protocols and be well described so that the intricacies of aerosol generation and measurement can be replicated by others. Indeed, the process of establishing constant and reproducible exposure conditions are considerably more complex than that required for portals of entry such as oral or dermal. This is a direct result of the type of equipment needed to generate, maintain, and measure experimentally produced atmospheres in a form that can be inhaled by the test species. Furthermore, there are inherent difficulties in measuring the dose; that is, relating the quantity of inhaled materials to that absorbed or retained in the test system. The total dose received depends on the physical and chemical properties of the material, the physiologic characteristics of the test animal, and the numerous factors involved in deposition and clearance. Thus it is clear that the technical difficulties in properly conducting an inhalation study are great.

Conditions exist in which the pulmonary effects of a chemical can not easily be evaluated by inhalation. Although not entirely valid reasons, space, time and/or economic reasons can sway the decision not to use inhalation as the route of test material exposure but rather to use intratracheal injection, a technique to get the material directly into the respiratory tract. The reason for choosing intratracheal instillation over inhalation, can also rest on non-availability of sufficient quantities of test material or on safety issues (extreme toxicity, flammability, explosivity). Using this method, the actual dose delivered to the lung of the experimental animal can be directly and precisely measured. This technique is inexpensive in that very small amounts of chemical are needed while expensive chambers, generating apparatus, and support personnel are avoided. Also, since the technique is contained and uses relatively little material, exposure hazards to laboratory workers are greatly reduced compared to that of an inhalation study. Finally, materials that are not readily respirable in rodents can be introduced to the lungs with this technique; notably, long fibers that can be inhaled by man but not by rodents can be tested via this route (Table 1). The problem that limits its usefulness of intratracheal instillation as an exposure technique relative to inhalation is that the dose to the respiratory tissues can be variable, highly artificial and does not accurately reflect the lung distribution of chemical following inhalation exposures (Table 2).

A note on terms - intratracheal instillation involves the introduction of particles in a carrier liquid by injection directly into the lining of the trachea or nebulized as very fine droplets into the airway. Intratracheal instillation is the same as the above without the use of a carrier liquid.

In intratracheal instillation, gravity cause the fluid and particles to flow into the dependant areas of the lung. The carrier liquid is then rapidly absorbed into the pulmonary circulation leaving the particles on the internal surfaces of the lung. This technique permits the introduction of a wide range of doses to the lung in a short period of time. In larger animals, localized exposures to specific areas or lobes of the lung can be administered, often allowing the contralateral lung to serve as a control (for non-systemically acting agents).

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The technique was first applied by Kimura[1] using rabbits and guinea pigs and looking at the response to various coal tars. The following early works describe in detail the methodology found useful in the mouse[2,3], in the rat[4], and in the hamster[5]. The procedures used in these studies are quite similar with the main difference in the maximal amount of total liquid that can be used to deliver the test agent to the lung without killing the animal.

Methods

In small rodents, intratracheal instillation is accomplished by inserting a catheter or needle transorally through the mouth and epiglottis into the tracheal lumen. In larger species (including man), a fiberoptic bronchoscope can be used to more precisely visualize the instillation site. Since the animal must not move during the procedure, the choice of anesthetic is important with short acting materials that suppress reflexes for a minimal period of time being preferred. Saline is the vehicle most frequently used to suspend or solubilize the test substance although even this may evoke a mild transient inflammatory response. Surfactants can be used to improve the suspension properties but the effect of the lung tissue needs to be considered. In addition, dosage volumes need to be adjusted for the body weight of the animal; some evidence suggests that larger volumes might distribute the agent more evenly in the lung however, excessive volumes will suffocate the animal. The rate of instillation must be controlled.

Parameters to Consider

Some advantages to this exposure system compared to inhalation methods include the ability to deliver an exact amount of material to the lung. As stated earlier, the procedure is considerably simpler, requires little equipment and technical support personnel, and is safer to attending personnel. One can deliver relatively large amounts of material to the target in a short period of time. Although the technique enables administration of large amounts and nonrespirable sizes of particulate that would otherwise not be able to gain access to the lung, highly localized deposition of particulate matter usually results. Indeed, the major obstacle for routine use of intratracheal instillation as a replacement for inhalation bioassays lies in the fact that the patterns of particle distribution in the lung following instillation are uneven and are unlike those resulting from inhalation. Particle deposition by inhalation is focal, that is, inhaled particles deposit at selected sites in the lung. Particles subsequently interact with complement proteins, which are components of the surface-lining layer of the distal lung, generating chemotactic factors as a by-product of this reaction[6]. These factors then serve to recruit pulmonary macrophages to the sites of particle deposition.

Applications

Brain and co-workers[7] showed that intratracheal instillation of particles produced nonuniform deposition patterns largely dependent on gravitational settling. These investigators studied the distribution of particles labeled with ^{99}Tc in both rats and hamsters following either intratracheal injection or aerosol inhalation. Particle distribution patterns in the lung following inhalation were distributed evenly in most of the deposited dust in the apical lobes[8]. More than simply nonuniform distribution, Pritchard[9] found that variability in the retention of cerium oxide particles in rats within a specific lobe was considerably greater following instillation than inhalation. Greater peripheral lung loading was seen following inhalation of ferric oxide particles than following instillation[10]. Using electron microscopic techniques, Brody and Roe[8] have shown that inhaled particles and fibers, which are small enough to pass through the conducting airways, deposit at selective sites (i.e., alveolar duct bifurcations) in the distal lung. This preferential deposition pattern has been confirmed by Warheit et al[11] in several rodent species and substantiates the idea that the initial distribution pattern of inhaled particles appears to be focal. In contrast, the distribution of both short and long glass fibers in rats was reportedly similar using either inhalation or instillation[12]. Drew[13] and Muller[14] found that both routes produced the same relative lobular distribution of uranium oxide particles. Despite these issues, other studies show comparable levels of pulmonary injury for instilled or inhaled quartz dust, although animals that inhaled quartz developed granulomas whereas the instilled animals did not[15].

Conclusions

As a consequence of these differences in cellular and biochemical reactions, intratracheal instillation is relatively nonphysiological approach with respect to the deposition patterns and can create an artefactual series of cellular (macrophage) reactions that do not accurately reflect the events that occur following inhalation exposure to dusts. Nonetheless, intratracheal instillation methods are being refined and used *in lieu* of formal inhalation studies for some applications. Recently, a series of studies were completed to validate intratracheal instillation methodology and its applicability to more traditional inhalation studies data. Rather than use an injected liquid bolus, liquid suspensions were injected using a bolus of air to form aerosol droplets within the airway. In this way, aerosol droplets were more uniformly distributed throughout the respiratory tract. These studies also indicated that particle size of the test suspension was not critical to the distribution of test particles into the lower respiratory tract and that this intratracheal nebulization method would be suitable for pulmonary absorption and disposition studies where knowing the precise dose administered is the primary concern[16]. Such methodology may find applications in acute toxicity screening studies or

mechanistic studies where inhalation exposure doesn't necessarily provide any advantages.

Evaluation of lung response to agents following intratracheal instillation (insufflation) needs to be done carefully. The investigator needs to properly qualify the results of such an exposure with the limitations of the technique recognizing it to be an alternative to inhalation. Proper perspective results from description of both the biological responses and the deposition profile. Comparison of the deposition following instillation and inhalation can extend/limit the real-world value of instillation methodology. The technique certainly has a place in hazard evaluation – the skilled and responsible investigator needs to apply the appropriate perspective to the obtained results.

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Table 1. Advantages of IT Technique.

Advantages of IT Technique
• Deliver exact amount of material to the lung (high local exposures possible)
• Procedure simple (vs. inhalation), little equipment needed, technical support personnel less critical
• Requires small amounts of test material
• Skin absorption confounders avoided
• Safety concerns - Work with small amounts of test material - Extreme toxicity, flammability, explosivity issues maximized
• Can test materials not readily respirable

Table 2. Disadvantages of IT Technique.

Disadvantages of IT Technique
• Dose to respiratory tissues can be variable
• Distribution/deposition of agents within respiratory tract do not reflect distribution following inhalation
• Can test materials not readily respirable