A Study to Investigate the Ability of Inhaled Amiodarone to Induce ‘Foamy’ Alveolar Macrophages in Male Wistar Han Rats

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Introduction

The differentiation of alveolar macrophages (AM) to a ‘foamy’ phenotype in toxicology studies often hinders inhaled drug entry into further clinical development [1]. The ‘foamy’ macrophage (FM) phenotype is a term used to describe the vacuolated appearance of an AM, seen by light microscopy, due to the presence of lamellar bodies, or an accumulation of lipids or drug particles in the cytoplasm of macrophages [1].

To improve inhaled drug development, a better understanding is required of FM biology.

Amiodarone is a prototypic cationic amphiphilic drug, well-known for its pulmonary toxicity profile and ability to induce a foamy phenotype in the AM population following systemic administration [2,3].

Aims

To assess lung macrophage responses following inhaled dry powder and oral administration of amiodarone in male Wistar Han rats as an exemplar of a drug known to induce FM.

Methods

Male Wistar Han rats (Charles River), were exposed to amiodarone by one of two routes:

- **Inhaled Dosing Using the Wrights Dust Feeder (WDF)**
  - Animals (250 - 300 g) were exposed for 30 min to air or a dry powder aerosol of micronised amiodarone (15 %) in a lactose blend (MMAD 2.1 µm and GSD 2.1 µm) using the WDF. A regulated flow of compressed air (~14L/min) delivered the aerosol from the WDF into an inhalation chamber. A slight draw (0.5 L/min) of room air into the chamber was allowed to balance the airflow and maintain the chamber at near ambient pressure.

- **Oral Administration of Amiodarone**
  - Rats of slightly larger body weight (300 - 395 g) were dosed orally with amiodarone (hydrochloride salt) 100 mg/kg, suspended in 1% w/v methylcellulose for 7 consecutive days and culled on day 1 post dosing.

Macrophase responses were evaluated using conventional histopathology (formalin fixed lungs were dehydrated through ascending grades of ethanol, paraffin wax embedded before H&E staining) and BAL assessment (total cells and differential cell counts performed using cytopsin preparations).

Results

Administration of amiodarone via the aerosol route did not induce an increase in total cells or macrophase influx across all time points (p > 0.05) (Figure 1A & B).

Inflammation was measured as neutrophil and eosinophil influx. The number of neutrophils and eosinophils were significantly elevated (* p < 0.05, ** p < 0.001) by administration of amiodarone with 10 mg/kg (day -3) and 30 mg/kg (days -2, 1 and 0) in the lungs of rats (Figure 1A & B).

Oral administration of amiodarone did not elicit an inflammatory response nor an increase in total cells (Figure 1A).

Histological analysis of lung tissue from the aerosol study showed no abnormalities in the lungs of air controls at day 1 and 7 post dosing (Figure 3).

Minimal to slight perivascular / peribronchiolar inflammatory cell infiltrate was observed in animals receiving aerosol amiodarone at day 1 and day 7 post doing (Figure 3).

Oral administration of amiodarone showed no significant pathology.

Discussion and Conclusion

Oral administration of amiodarone induced a more robust FM response of both finely and coarsely vacuolated macrophages with no inflammatory response at day 1 post dosing.

Finely vacuolated AM may be associated with apoptosis [4], whereas, the coarsely vacuolated AM phenotype is reported to be indicative of autophagy [5].

Inhaled amiodarone caused transient pulmonary inflammation with little effect on AM vacuolation. This suggest that the amiodarone FM effect is systemic and inhaled amiodarone cannot be used as a positive control for inducing FM.

References


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