

BACKGROUND

Few new inhaled therapies have reached the market in the past 30 years, in part to a lack of understanding of how the airways respond to inhaled particulate therapies¹. A highly vacuolated alveolar macrophage response is often observed in pre-clinical studies in the lungs of rats when dosed with inhaled particulate drug compounds, however it is unknown if these responses are a result of drug pharmacology or an interaction with the insoluble particulates in the lung^{1,2}. Amiodarone was selected as a model drug as it is established for inducing phospholipidosis in the airways when administered orally and possesses very poor water solubility (1.1 mM at 25 °C in water and up to 50 mM in DMSO)³.

AIM

To develop *in vitro* methodology to assess if cellular responses to poorly aqueous soluble compounds were caused by the pharmacology of the drug and the soluble fraction entering the cells, or by physicochemical interaction of the insoluble particulate material with the alveolar macrophages.

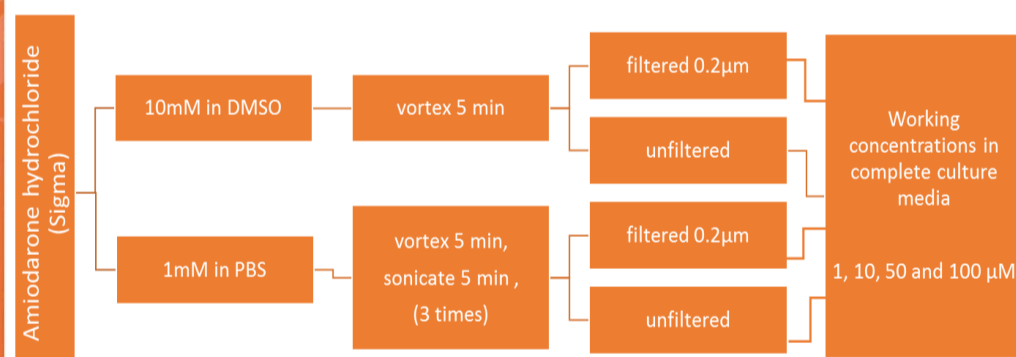
METHODOLOGY**1. Compound preparation**

Figure 1: Amiodarone hydrochloride preparation scheme.

2. Analysis of amiodarone solutions

A Perkin Elmer liquid chromatographic system (Coventry, West Midlands, UK) with a Flexar UV/VIS Detector was used for separation. A detection wavelength was 244 nm. The particle size and charge of amiodarone solutions was measured using a ZetaSizer (Malvern Instruments, Worcestershire, UK).

3. Cell culture: viability and phospholipid assessment

Rat macrophage cells, NR8383 were seeded into 96-well plates at a density of 3×10^4 cells/well and exposed to various amiodarone concentrations (1, 10, 50, 100 µM) prepared as outlined above, for 24, 48 and 72 hours. Flow cytometry was used to assess cell viability (Guava ViaCount kit according to manufacturer's protocol, Millipore) or the accumulation of phospholipids (Nile Red staining technique as previously described⁴).

REFERENCES

- 1) Forbes B, et al. *Advanced drug delivery reviews*. 2014;71:15-33
- 2) Lewis DJ, et al. *J Appl Toxicol*. 2014;34:319-331
- 3) Reasor MJ et al. *Exp Biol Med (Maywood)*. 2001;226:825-830
- 4) Casartelli A et al. *Cell biology and toxicology*. 2003;19:161-176

RESULTS

Unfiltered Amiodarone Preparation		Filtered Amiodarone Preparation	
Theoretical Concentration (µM)	Actual Concentration (µM)	Theoretical Concentration (µM)	Actual Concentration (µM)
1	1.8	1	1.8
10	8.7	10	9.3
50	55.2	50	54.0
100	103.8	100	95.3

Table 1: Concentration of amiodarone in working solutions determined using HPLC.

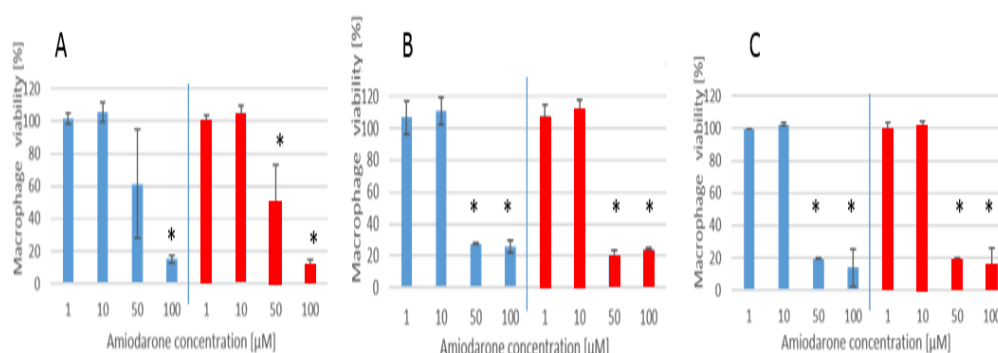


Figure 2: Cell viability of NR8383 treated with filtered and unfiltered preparations of amiodarone for (A) 24 h, (B) 48 h and (C) 72 h. Data is presented as mean \pm SD of cell viability relative to untreated cells of three (n=3) experiments. Blue bars indicate amiodarone preparations filtered through a 0.2 µm filter, red bars indicated unfiltered solutions. * indicates $p < 0.05$ in comparison with untreated control.

Unfiltered Amiodarone Preparation				Filtered Amiodarone Preparation			
Concentration (µM)	Nile Red Staining			Concentration (µM)	Nile Red Staining		
	24h	48h	72h		24h	48h	72h
1	-	-	-	1	-	-	-
10	+	+	+	10	-	++	+
50	+	++	+	50	+	++	+
100	++	++	++	100	++	++	++

Table 2: Determination of phospholipidosis and extent of vacuolation in NR8383 treated with unfiltered and filtered amiodarone preparations. Results are presented as a relative to untreated cells. - indicates no change in granularity and phospholipid accumulation; + 3 – 5% of cells showed increase in granularity and phospholipid accumulation; ++ 6 – 15% of cells showed increase in granularity and phospholipid accumulation.

CONCLUSION

- No significant difference ($p > 0.05$) was observed in cell viability or phospholipidosis between filtered and unfiltered preparations of amiodarone.
- HPLC analysis of the concentrations of amiodarone in filtered and unfiltered solutions were the same in concentrations below 50 µM. At the highest concentration tested, a difference of 8.5 µM was observed between filtered and unfiltered solutions suggesting that approximately 8% of the amiodarone in the unfiltered preparation was suspended as particulates greater than 0.2 µm in size.
- These results suggest that *in vitro*, amiodarone toxicity is primarily mediated by the drug in its solubilised form and is less related to the physicochemical properties of the insoluble particulate fraction.