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Rapid Communication

Technetium-99m labelling of suspension type pressurised metered dose inhalers comprising various drug/surfactant combinations

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Summary

The aerosol distribution, as assessed by cascade impaction, of ^{99m}Tc relative to drug and surfactant has been determined for various commercial formulations of pressurised metered dose inhalers (MDIs), labelled by two established methods. The location of the radiolabel (i.e. drug or surfactant-associated) was independent of the labelling method but highly dependent on the surfactant/drug combination within the MDI.

For many years, the actual regional deposition pattern within the lung of aerosols emitted from pressurised metered dose inhalers (MDIs) was unknown. Recently, however, the technique of gamma scintigraphy has been used to provide valuable information about the extent and anatomical location of lung deposition of inhaled therapeutic aerosols. There are severe technological problems associated with the direct labelling of drugs with a gamma emitting radioisotope, and to date this has only been undertaken successfully with ipratropium [^{77}Br]bromide (Spiro et al., 1984). Indirect labeling procedures have thus been adopted, and the formulation of MDIs containing either model particles (^{99m}Tc-labelled teflon microspheres; Newman et al., 1981) or drug/radionuclide co-precipitates (Vidgren et al., 1987, 1988) has been reported. Radiolabelling of commercial MDI products was first investigated by Kohler et al. (1988) who reported the labelling of Berotec® (fenoterol) by addition of pertechnetate (^{99m}Tc-O_4^-) plus additional trichlorofluoromethane and sorbitan trioleate to facilitate dissolution of the radiochemical. This technique has been shown retrospectively to be highly variable and, furthermore, alters the particle size distribution of the aerosol compared to that of an unlabelled preparation (Summers et al., 1990). Two subsequent modifications to Köhler's method have been de-

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scribed that yield formulations preserving the original aerosol size distribution following actuation, as assessed with a multi-stage liquid impinger (MLI). Both methods involve addition of ^{99m}Tc, following extraction from chloroform as a complex with tetraphenylarsonium chloride (TPAC) (Newman et al., 1990, 1991) or as TcO_4^- from butan-1-one (Summers et al., 1990), to the pressure pack contents without co-addition of other non-volatile components. Despite observations that the aerosol size distributions of the drug and radiolabel are similar, no studies have been undertaken to demonstrate conclusively the mechanism of association between the drug particles and the radiochemical. Köhler et al. (1988) suggested that there was "probably simple solution of $^{99m}TcO_4^-$ in the polar β_2 agonist". Since this would involve displacement of a pre-adsorbed surfactant envelope it would appear more likely that the label is in some way associated with this adsorbed layer. Evans et al. (1988) demonstrated that low HLB surfactants of the type used in a typical suspension type MDI formulation can self-associate to different extents within CFCs (lecithin > sorbitan trioleate ⋙ oleic acid). Aggregation and adsorption may be considered as similar energetic processes (Couper et al., 1975) and thus the propensity for adsorption onto micronised drug particles may well depend on which process is the more energetically favourable. We may therefore predict that with different drug/surfactant combinations the location of the gamma label may well be determined by the thermodynamics of the system.

In order to test this hypothesis, we have attempted to compare the distribution of ^{99m}Tc within three commercial, CFC-based suspension systems, and in model 'aerosol' systems having similar drug concentration and drug:surfactant weight ratios to the actual MDIs. The MDIs evaluated were Ventolin® (salbutamol base/oleic acid), Alupent® (orciprenaline sulphate/soya lecithin) and Intal® (sodium cromoglycate/sorbitan trioleate). 25 MBq of ^{99m}Tc-TPAC (method I; Newman et al., 1990) or $^{99m}TcO_4^-$ (method II; Summers et al., 1990, except that acetone instead of butan-1-one was employed as the organic solvent) was transferred to an empty aluminium aerosol canister. 74 kBq of the appropriate ^{14}C-labelled surfactant (Amersham International, U.K.) was added next and residual solvent evaporated to dryness with nitrogen. Following removal of the valve assembly from an MDI unit cooled to $-60°C$, the contents were transferred to the labelled canister and a new metering valve crimped into position. The sealed canister was mechanically agitated for 1 h. Previous studies have indicated the appropriateness of ^{14}C-labelled analogues as markers of surfactant in CFC-based suspensions (Clarke et al., 1990) and the agitation time was considered sufficient for re-equilibration, which is known to be rapid for adsorption phenomena on non-porous crystalline materials (Sato and Ruch, 1980). Each canister was actuated five times to prime the valve which was then cleaned prior to fitting an oral adaptor and securing the inverted canister at the entrance to a glass 'throat' on top of an eight-stage Andersen cascade impactor. In vitro aerosol size analysis was carried out using an air flow of 28.3 l min^{-1}. The number of actuations necessary depended on the drug assay sensitivity and was found to be five with Intal, 20 with Alupent and 40 with Ventolin. Non-volatile components of the aerosol cloud were quantitatively washed off the oral adaptor, throat and various impactor stages using 50% (Intal) or 60% (Ventolin and Alupent) aqueous ethanol. Aliquots were immediately taken for detection of ^{99m}Tc (LKB Compugamma counter), and following a 48 h decay period for drug by UV spectrophotometry (276 nm for salbutamol and orciprenaline; 326 nm for sodium cromoglycate) and ^{14}C-surfactant by liquid scintillation counting (LKB RackBeta). The beta-emitting daughter isotope of ^{99m}Tc (^{99}Tc) showed no interference with ^{14}C detection. The model 'aerosol' suspensions containing the drug, surfactant and 5 MBq of the gamma-emitting radiochemical were prepared in an *n*-hexane:trichlorotrifluoroethane (50:50) solvent system, which ensured ready separation of the drug with adsorbed surfactant by bench centrifugation at 2000 rpm for 30 min. This solvent system also displays a similar dielectric constant (2.1) to those of conventional propellant blends. The dispersions were 'tumble-mixed' for 1 h,

after which they were centrifuged and aliquots of the clear supernatant taken for gamma counting. The percentage associated with the drug and adsorbed surfactant was calculated by difference from the percentage of original activity remaining in solution.

In Table 1, the relative distribution of each of the non-volatile components emitted from the various MDIs under investigation is represented as the cumulative proportion deposited within the oral adaptor and throat (i.e., extrapulmonary deposition) and the proportion located on the various stages of the impactor. The respirable fraction (RF) is calculated from aerosol deposited on the six later stages of the device, i.e., droplets with aerodynamic diameters < 5.8 μm. For all the MDI formulations studied, there was no significant difference between the non-volatile components for both extrapulmonary and impactor deposition (one-way ANOVA and post-hoc Duncan's multiple-range test), whereas there was deviation between the RF values for drug, surfactant and gamma radiolabel. This is further demonstrated by comparison of size distribution data for the three non-volatile components present in the MDI formulations labelled by either technique (Fig. 1). The graphs show that in all cases, size separation of the drug and surfactant in the impactor was observed, with the surfactant droplet size consistently smaller than that of the drug. This phenomenon has been previously reported for Ventolin (Malton et al., 1982); Callingham (1980) has provided a model to account for the disproportionation by size during atomisation of propellant-insoluble (e.g. drug) and propellant-soluble (e.g. surfactant) non-volatiles. Irrespective of labelling technique, the gamma label does act as a marker for drug distribution in the case of Ventolin and Intal MDIs. Evidence for association of ^{99m}Tc with the dispersed drug in each case

TABLE 1

Deposition of non-volatile components (mean ± SD, n = 3) emitted from MDIs as a function of formulation and method of radiolabelling

	Method I			Method II		
	Actuator/ Throat	Impactor	RF	Actuator/ Throat	Impactor	RF
Alupent						
Drug	78.93	21.07	13.88	83.43	16.57	11.78
	(1.18)	(1.18)	(1.31)	(1.08)	(1.08)	(0.91)
Surfactant	73.98	26.02	18.71	80.28	19.72	14.89
	(1.01)	(1.01)	(1.10)	(0.99)	(0.99)	(1.25)
Technetium	76.85	23.14	16.66	82.40	17.60	13.06
	(1.29)	(1.28)	(1.52)	(0.95)	(0.95)	(1.51)
Intal						
Drug	84.12	15.88	6.23	82.30	17.70	10.05
	(2.48)	(2.48)	(0.92)	(1.65)	(1.65)	(0.91)
Surfactant	82.61	17.38	8.80	78.76	21.24	13.67
	(2.80)	(2.79)	(1.26)	(1.07)	(1.07)	(0.93)
Technetium	85.69	14.31	5.68	83.34	16.66	8.05
	(2.17)	(2.17)	(1.03)	(1.61)	(1.61)	(2.04)
Ventolin						
Drug	74.08	25.91	23.19	69.99	30.01	28.88
	(1.91)	(1.91)	(1.89)	(2.34)	(2.34)	(2.31)
Surfactant	75.36	24.64	23.34	71.50	28.50	27.74
	(5.63)	(5.63)	(5.65)	(1.54)	(1.54)	(1.48)
Technetium	77.13	22.87	19.47	73.82	26.18	25.23
	(2.39)	(2.39)	(0.92)	(0.76)	(0.76)	(0.66)

is provided by the fact that as droplet size decreases the relative depositions of both drug and ^{99m}Tc decrease but that of surfactant increases. This was further confirmed in the model aerosol systems, where, respectively, 98.5 and 99.3% of ^{99m}Tc was associated with the centrifuged plug of drug. The converse was true, however, for Alupent MDIs where ^{99m}Tc activity appears to follow the surfactant distribution. In model Alupent systems, only 5.6% of the gamma radioactivity centrifuged with the drug. Lecithin has a high tendency to aggregate in CFCs (Evans et al., 988), forming reverse micellar structures composed of a large number of monomers (Evans et al., 1990). The aqueous nature of these micellar cores within the bulk propellant blend probably provides a more favourable location for the gamma label over the drug particle surface. Conversely, the aggregation tendency of oleic acid in a CFC environment is extremely weak (Evans et al., 1988), suggesting that the disperse phase in Ventolin offers a more energetically favourable environment for the gamma label than the apolar CFC blend. Sorbitan trioleate can also self-associate in CFCs, albeit to a lesser extent than lecithin, although the size and shape of the resultant micelles have not been characterised. Should these micelles contain only few monomers, as is normal for surfactants in apolar media (Eicke, 1980), this might explain the preferential association of ^{99m}Tc with the drug rather than the surfactant in the Intal formulation.

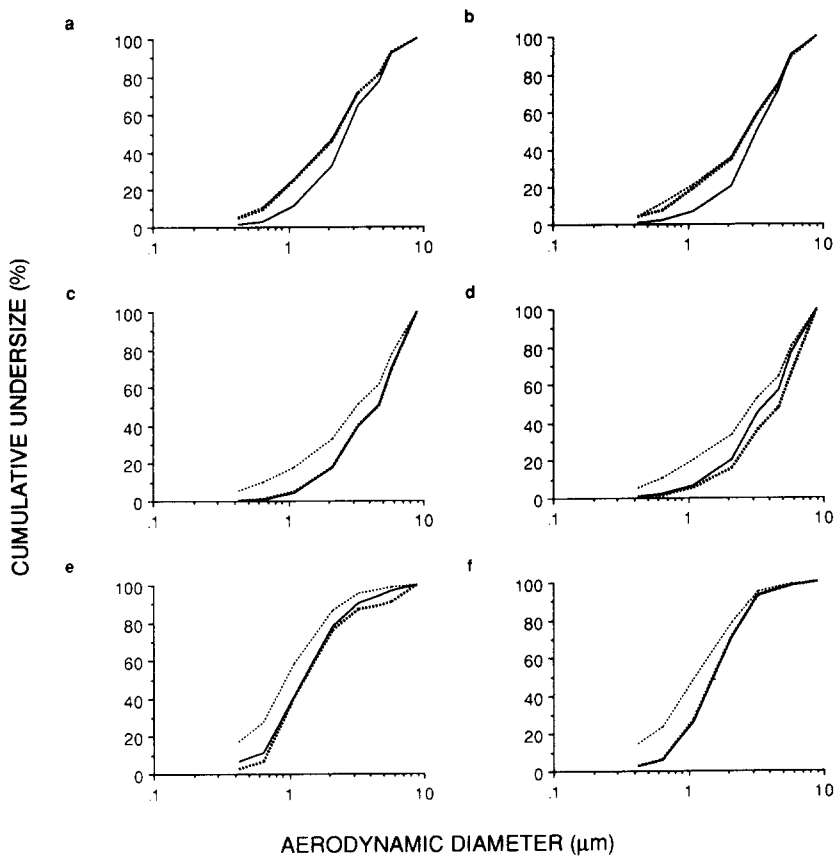

Fig. 1. Relative size distribution of drug, surfactant, and gamma radiolabel deposited on eight stages of an Andersen impactor as a function of MDI formulation and radiolabelling method. Panels a, c, e and b, d, f refer to MDIs labelled by method I and method II, respectively. (a, b) Alupent, (c, d) Intal, (e, f) Ventolin. Distribution profiles: (———) drug, (· · · · · ·) surfactant, (— — —) ^{99m}Tc.

These results strongly suggest that the location of a ^{99m}Tc radiolabel will be determined by the presence and nature of surfactant reverse micelles, and the character of the surface of the disperse phase. Which process (i.e., surfactant-mediated solubilisation within the CFC blend or association with drug and adsorbed surfactant) occurs will depend on which is thermodynamically more favourable. It is obvious from this series of experiments that the labelling technique is not generally applicable to all MDI products, and that careful in vitro validation is an essential prerequisite to undertaking in vivo deposition studies. In this respect, it is preferable to employ a cascade impactor with a large number of collection stages (such as the Andersen eight-stage device) so that the aerosol size distribution of the various non-volatile components can be closely defined. A formulation in which the gamma radiolabel follows the surfactant distribution, as in the case of Alupent, could be translated in vivo into an erroneously high drug deposition in the lung, particularly in relation to the alveolar deposited fraction.

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