Surface Active Agents as Enhancers of Alveolar Absorption

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Purpose. Small solutes which are deposited in the alveoli by aerosol inhalation will be absorbed across the alveolo-capillary barrier. Inhalation of dioctyl sodium sulfosuccinate (DOSS) enhances absorption while having little or no effect on lung function, suggesting that surface active agents may be used as enhancers of alveolar absorption of inhaled pharmaceuticals. The purpose of this study was to examine the effects of a selection of different surface active agents on alveolar absorption.

Methods. The absorption of ^{99m}Tc-diethylene triamine pentaacetate (^{99m}Tc-DTPA) from the lungs was studied in rabbits. We studied five different surface active agents: DOSS, sodium glycodioxycholate (GDCA), sodium lauryl sulphate (NaLS), lysophosphatidyl choline (LPC) and polyoxyethylene-23-laurylether (P23LE).

Results. DOSS and GDCA both dramatically enhanced the absorption of ^{99m}Tc-DTPA. There was a moderate effect of NaLS, no significant effect of LPC and P23LE reduced the rate of absorption. None of the compounds affected gas exchange or lung compliance.

Conclusions. There is a wide spectrum of effects of inhaled surface active agents on the alveolar absorption of ^{99m}Tc-DTPA. Ionic compounds such as DOSS and GDCA have the greatest effect, and further studies of these classes of surface active agents for use as enhancers of alveolar absorption of pharmaceuticals seem warranted.

KEY WORDS: absorption; aerosols; surface active agents; drug administration routes; drug formulation; radioactive imaging.

INTRODUCTION

Low molecular weight solutes which are deposited in the alveoli by aerosol inhalation will be absorbed to the blood across the alveolo-capillary barrier. In addition to the alveolar epithelial cell and the capillary endothelial cell, the alveolar surfactant system forms a barrier against solute absorption (1). The alveolo-capillary transfer of radiolabelled solutes has been studied extensively in health and disease, most often using ^{99m}Tc-diethylene triamine pentaacetate (^{99m}Tc-DTPA) as an experimental tracer molecule (1–2). If surfactant dysfunction is induced by administration of the surface active agent dioctyl sodium sulfosuccinate (DOSS) in aerosol form, the rate of absorption of ^{99m}Tc-DTPA increases dramatically (3–5). If, on the other hand, the alveolar content of surfactant is increased, the rate of absorption is reduced (6).

The mechanism by which the surface active agent increases the alveolo-capillary transfer of solutes is not clear. The compound may interact with surfactant phospholipids at the molecular level to change the properties of the surfactant system. It may also affect the alveolar epithelial cell, e.g., by making the tight junctions more permeable.

Administration of a surface active agent may increase the rate of absorption of ^{99m}Tc-DTPA dramatically while having little or no effect on gas exchange or lung compliance (3–5). This suggests that surface active agents may be used as enhancers of absorption in pharmaceutical preparations for inhalation. A recent study has also shown that addition of DOSS to a powder aerosol of insulin resulted in rapid absorption of insulin, peak plasma concentration being reached within 30 min (Almér *et al.*, submitted for publication). Although DOSS was well tolerated in this study, it is probably not an ideal substance. It has a waxy texture and is difficult to mix in pharmaceutical powder preparations.

In this study, we have examined the effects of a range of different surface active agents on the alveolo-capillary transfer of ^{99m}Tc-DTPA with the purpose of providing a better basis for understanding of the effects of surface active agents as enhancers of alveolar absorption and a basis for selection of surface active agents that may be suitable in pharmaceutical preparations.

MATERIAL AND METHODS

Animal Preparation

Experiments were performed in rabbits, which were anaesthetised with an i.v. injection of thiopental sodium (30 mg/kg), followed by an intravenous infusion to maintain anaesthesia. The animals were tracheotomised and ventilated with a Servo Ventilator 900C (Siemens-Elelma, Solna, Sweden) in the pressure controlled mode. End-tidal $PCOD_2$ was monitored with a CO_2 analyser (Siemens-Elema AB), and peak inspiratory pressure was adjusted to keep end-tidal PCO_2 at approximately 4 kPa. This resulted in a peak inspiratory pressure of approximately 12 cmH_2O. A positive end-expiratory pressure (PEEP) of 2 cmH_2O was maintained to prevent atelectasis. Ventilator settings were kept constant during the experiments. Muscle relaxation was obtained with pancuronium bromide. A femoral artery was cannulated for blood sampling.

Experimental Protocol

A solution of ^{99m}Tc-DTPA was nebulised with an Ultra Vent nebuliser (Mallinckrodt Diagnostica, Petten, the Netherlands). The aerosol was generated during expiration, filling the inspiratory line of the ventilation circuit. The particles were then administered with the following insufflation (7). With this mode of administration, airway pressures are virtually unchanged during aerosol administration (7) and the particles are carried to the alveolar region of the lungs (8). Radioactivity was measured over the chest of the rabbit with a gamma camera (Portacamera, General Electric Co, Milwaukee, Wis) equipped

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ABBREVIATIONS: ANOVA, analysis of variance; C_{rs}, compliance of the respiratory system; DOSS, dioctyl sodium sulfosuccinate; DTPA, diethylene triamine pentaacetate; GDCA, sodium glycodioxycholate; LPC, lysophosphatidyl choline; NaLS, sodium lauryl sulphate; PCO_2, partial pressure for carbon dioxide; PEEP, positive end-expiratory pressure; PO_2, partial pressure for oxygen; P23LE, polyoxyethylene-23-laurylether.

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with a converging collimator. Radioactivity was recorded in 1 min frames for 90 min, starting immediately after administration of ^{99m}Tc-DTPA. The first 30 min constituted a control period, during which the basal rate of alveolo-capillary transfer of ^{99m}Tc-DTPA was measured. After 30 min, a 0.2% solution of a surface active agent was nebulised as described above. The aerosol containing the surface active agent was always administered for 5 min, resulting in deposition of approximately 10 μl of fluid in the lungs (4).

Radioactivity was recorded for another 30 min. A second dose of surface active agent (2% solution) was administered at 60 min, and radioactivity recorded for another 30 min. A few minutes before the end of the registration, a small amount (approximately 5 MBq) of ^{99m}Tc-DTPA was injected intravenously for background correction (9). This procedure corrects for tracer recirculating in the systemic circulation, e.g., in the chest wall.

Arterial blood samples were drawn before, 30 min, 60 min, and 90 min after the administration of ^{99m}Tc-DTPA. Arterial PO_2 and PCO_2 were immediately measured with a blood gas analyser (ABL300, Radiometer A/S, Copenhagen, Denmark). At the same times, readings of end-inspiratory and end-expiratory pressure as well as tidal volume were obtained from the ventilator. The compliance of the respiratory system (C_{rs}) was calculated as tidal volume divided by the difference between end-inspiratory pressure and PEEP and normalised to body weight. C_{rs} provides an index of the distensibility of the lungs. Groups of six or seven animals were studied for each surface active agent.

Data Analysis

The retention curves recorded over the lungs were analysed by fitting a mono-exponential equation to the experimental data in the time intervals 0–30 min, 30–60 min and 60–90 min. The half-life of ^{99m}Tc-DTPA in the lungs was then calculated.

Surface Active Agents

The following surface active agents were investigated: DOSS, sodium glycodioxycholate (GDCA), sodium lauryl sulphate (NaLS), lysophosphatidyl choline (LPC) and polyoxyethylene-23-laurylether (P23LE). DOSS was dissolved in 50% ethanol and 50% isotonic saline. This vehicle containing ethanol does not itself affect the alveolo-capillary transfer of ^{99m}Tc-DTPA (3–5). The other surface active agents were dissolved in isotonic saline.

Statistical Analysis

Data are presented as means ± s.d. Between groups differences at the start of the experiment were analysed with Kruskal-Wallis ANOVA. Within group changes after administration of the surface active agent were analysed with Friedman's ANOVA.

RESULTS

The half-life of ^{99m}Tc-DTPA in the lungs during the control period and after administration of the two doses of surface active agent are presented in Table 1. There were no significant differences between the groups during the control period. Administration of surface active agent resulted in significant reduction in the half-life of ^{99m}Tc-DTPA for DOSS, GDCA and NaLS (Friedman's ANOVA, $p < 0.01$ for all agents). There was no significant change for LPC ($p = 0.14$), and the half-life was prolonged by administration of P23LE ($p < 0.05$). Fig. 1 shows the half-lives after administration of surface active agent normalised to the half-life during the control period, so as to better demonstrate the relative effect.

Table 1. Half-Life of ^{99m}Tc-DTPA in the Lungs After Treatment with the Different Detergents

	Half-life (min)		
	Control	0.2% detergent	2% detergent
DOSS	111 ± 16	34 ± 5	11 ± 1
GDCA	94 ± 16	39 ± 11	15 ± 2
NaLS	116 ± 8	64 ± 16	19 ± 2
LPC	108 ± 24	106 ± 27	99 ± 23
P23LE	122 ± 16	141 ± 20	151 ± 24

Note: DOSS = sodium dioctyl sulfosuccinate, GDCA = sodium glycodioxycholate, NaLS = sodium lauryl sulphate, LPC = lysophosphatidyl choline, P23LE = polyoxyethylene-23-laurylether.

There were no between group differences in blood gases or dynamic compliance at the start of the experiment (Table 2), and no significant changes after administration of surface active agent in any of the groups.

DISCUSSION

A large number of studies over the last decade using radiolabelled tracers have revealed that absorption of inhaled solutes across the alveolo-capillary barrier is quite variable (1–2). ^{99m}Tc-DTPA, which is the most widely used tracer, is very hydrophilic and has a relatively low molecular weight (492 dalton). Physiologically, the rate of absorption of ^{99m}Tc-DTPA varies with the breathing pattern, increasing with lung volume (10–11). In smokers, the rate of absorption is greatly increased and related to the amount of alveolar surfactant (12–13). Absorption of lipophilic solutes appears to be little affected by the surfactant system (14). The rate of absorption of radiolabelled albumin is increased in a qualitatively similar way as

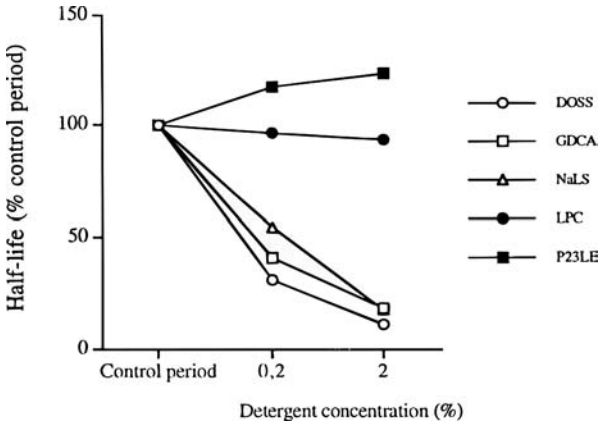

Fig. 1. Half-life of ^{99m}Tc-DTPA in the lungs before and after detergent administration. The half-life after detergent administration has been normalised to the half-life during the control period.

Table 2. Blood Gases and Compliance of the Respiratory System (C_{rs}) Before and After Aerosol Administration

	Before ^{99m}Tc-DTPA	Time after ^{99m}Tc-DTPA		
		30 min	60 min	90 min
PO_2 (kPa)				
DOSS	10.4 ± 0.8	10.6 ± 0.4	9.6 ± 0.9	11.4 ± 1.0
GDCA	10.3 ± 0.8	9.8 ± 1.1	9.8 ± 0.8	8.5 ± 0.8
NaLS	11.5 ± 0.7	11.3 ± 0.6	10.6 ± 0.7	9.8 ± 1.1
LPC	10.7 ± 1.0	10.0 ± 1.0	11.4 ± 0.6	9.9 ± 1.3
P23LE	9.0 ± 0.9	10.0 ± 0.3	10.2 ± 0.8	9.7 ± 0.4
PCO_2 (kPa)				
DOSS	3.8 ± 0.3	3.5 ± 0.2	4.2 ± 0.3	4.1 ± 0.4
GDCA	3.7 ± 0.3	4.3 ± 0.4	4.8 ± 0.6	5.2 ± 0.7
NaLS	3.7 ± 0.2	3.6 ± 0.2	3.6 ± 0.2	4.0 ± 0.4
LPC	3.9 ± 0.2	4.1 ± 0.4	3.6 ± 0.4	3.9 ± 0.4
P23LE	4.7 ± 0.6	4.2 ± 0.2	4.3 ± 0.3	4.4 ± 0.2
C_{rs} (ml min^{-1} kg^{-1})				
DOSS	1.03 ± 0.07	1.03 ± 0.09	1.01 ± 0.08	1.15 ± 0.10
GDCA	1.00 ± 0.06	1.03 ± 0.07	0.98 ± 0.07	0.95 ± 0.06
NaLS	1.20 ± 0.10	1.11 ± 0.07	1.07 ± 0.05	1.21 ± 0.17
LPC	1.00 ± 0.06	1.03 ± 0.05	1.07 ± 0.05	1.06 ± 0.05
P23LE	1.03 ± 0.05	1.03 ± 0.06	1.05 ± 0.05	0.97 ± 0.10

Note: Detergent aerosol was administered 30 min (0.2 % solution) and 60 min (2 % solution) after ^{99m}Tc-DTPA. DOSS = sodium dioctyl sulfosuccinate, GDCA = sodium glycodioxycholate, NaLS = sodium lauryl sulphate, LPC = lysophosphatidyl choline, P23LE = polyoxyethylene-23-laurylether.

^{99m}Tc-DTPA during ventilation with large tidal volume (15) and in smokers (16). Rapid alveolo-capillary transfer of ^{99m}Tc-DTPA could predict rapid absorption of terbutaline from the lungs of smokers (17) and during exercise with increased ventilation (18). Measurement of the alveolo-capillary transfer of ^{99m}Tc-DTPA may therefore be a valuable model situation, in which factors influencing the alveolar absorption of pharmaceuticals can be assessed.

In the normal rabbit lung (4), as in the normal human lung (12), the alveolo-capillary transfer of ^{99m}Tc-DTPA follows a mono-exponential course over three hours. This allows multiple doses of surface active agent to be tested against an initial control period. The first 30 min after administration of ^{99m}Tc-DTPA in this study thus represent the basal rate of alveolo-capillary transfer of ^{99m}Tc-DTPA. In this preliminary study, we selected to use two doses of each surface active agent. The effect of DOSS lasts approximately two hours (4), and a carry-over effect of the low dose is likely to be present after administration of the higher dose.

Surface active agents are well known to increase absorption of pharmaceuticals across many epithelia (19) and have been evaluated as enhancers for nasal administration of insulin (20). DOSS has also been shown to increase the absorption of ^{51}Cr-ethylene diamine tetraacetate across the human nasal mucosa (21). In that study, a solution of 0.6% DOSS was kept in contact with the nasal mucosa for 15 min. At the alveolar level, as little as 10 μl of a 2.0% solution distributed over the whole alveolar surface in a rabbit results in dramatically increased absorption of ^{99m}Tc-DTPA (4). Although it is difficult to estimate the concentration of DOSS on the alveolar surface, it seems obvious that considerably lower concentrations of DOSS are required to increase alveolar than nasal absorption. A likely explanation for this difference is that the surface active agent interacts with surfactant phospholipids at the alveolar level, whereas the higher doses required in the nose affect the epithelial cells. An effect of DOSS on the alveolar epithelial tight junctions can, of course, not be ruled out.

The precise mechanism for interaction between surface active agents and surfactant is not known. In this study, we selected a range of compounds with widely different properties. DOSS, GDCA and NaLS are all negatively charged. LPC is a zwitterion and P23LE is not charged. DOSS is the only surface active agent with two carbon chains; GDCA is a steroid and the remaining compounds have single carbon chains. The relative size of the hydrophilic and hydrophobic part of the molecule is thus different for the selected surface active agents. The tendency for the compounds to form micelles is also different. The critical micelle concentration is very low for LPC and P23LE (approximately 10^{-3} and 10^{-1} mM, respectively (22,23)) compared to DOSS and NaLS (approximately 3 and 8 mM, respectively (24,25)). GDCA, being a steroid, does not form micelles in the traditional sense. Instead GDCA, like DOSS, has a tendency to form lamellar structures.

All ionic surface active agents in this study resulted in greatly increased alveolo-capillary transfer of ^{99m}Tc-DTPA. The effect of the zwitterionic LPC was intermediate, and the non-ionic P23LE actually reduced the alveolo-capillary transfer of ^{99m}Tc-DTPA. This may indicate that an ionic surface active agent has a greater propensity to interact with surfactant phospholipids. The most common surfactant phospholipid, dipalmitoyl phosphatidylcholine, is a zwitterion. Of the three ionic surface active agents, DOSS and GDCA had the largest effect. These surface active agents also have a tendency to form lamellar structures rather than micelles. This property may imply a greater tendency to interaction with the surfactant monolayer in the alveoli.

Judging from the studies of radiotracers with different characteristics (3,4,6,12,13,14,15,16) absorption enhancement

by administration of surface active agents may be applicable mainly to hydrophilic substances. The effect can be expected to be greatest on small molecules, but extends to as large molecules as albumin. There appears to be little, if any, effect on the absorption of lipophilic substances.

None of the surface active agents investigated in this study had any effect on gas exchange or dynamic compliance. This is in agreement with previous studies of the effect of DOSS (3–5) and indicates that acute administration of surface active agents has a potential to increase absorption of inhaled pharmaceuticals without having negative effects on lung function. Further work is clearly needed to establish if there are any adverse effects of repeated administrations of surface active agents. Possible effects include lung water accumulation and development of atelectasis. Such effects may to some extent have been ameliorated by the application of PEEP in this study.

In conclusion, this study has shown that there is a wide spectrum of effects of inhaled surface active agents on the alveolo-capillary transfer of ^{99m}Tc-DTPA. Ionic surface active agents such as DOSS and GDCA have the greatest effect, and further studies of these classes of surface active agents for use as enhancers of alveolar absorption of pharmaceuticals seem warranted.

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