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Research Article

Solute Absorption from the Airways of the Isolated Rat Lung. II. Effect of Surfactants on Absorption of Fluorescein[1]

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To determine the effects on the pulmonary barrier of several surface active agents, a series of metered dose inhalers (MDIs) was prepared and used to dose aerosolized surfactant to the airways of isolated perfused rat lungs. The MDIs contained a range of concentrations, from 0.1 to 5.0% (w/w), of either oleic acid, oleyl alcohol, or Span 85, which released ≈45 μg (0.1%, w/w) to ≈1660 μg (5.0%, w/w) of surfactant per actuation. The permeability of the pulmonary barrier was assessed by the rate of transfer of disodium fluorescein dosed as 100 μl of aqueous solution (1 mg/ml) after administering the surfactants. Some 12.1 ± 4.7% of the recovered surfactant, per dose, was assessed to reach the pulmonary regions of the lung. All surfactants tested caused an increase in fluorescein transfer rates. A single actuation from the MDI containing 5% (w/w) oleic acid produced gross edema in all lungs tested within 40 min and the first-order half-lives of absorption were reduced almost threefold, from 12.9 ± 2.5 min for controls to 4.5 ± 0.8 min. Differences in absorption were noted between the acid and the alcohol, which is consistent with the hypothesis that both the hydrocarbon chain and the polar head group have roles in the altered permeability to fluorescein. The absorption of fluorescein when dosed from the MDI containing 5% (w/w) Span 85 was increased but all surfactants dosed from the lowest concentration MDI of 0.1% (w/w) did not alter absorption rates of the dye relative to those of controls. Results are discussed in light of current interest in absorption enhancement and the presence of surfactants in currently marketed MDIs.

KEY WORDS: absorption; aerosol; epithelium; fluorescein; isolated lung; pulmonary barrier; surfactants.

INTRODUCTION

The use of surface active agents to promote drug absorption across membranes is of current interest and is seen to be successful in increasing drug permeability through barriers such as the skin (1–4), gastrointestinal tract (5–8), and nasal membranes (9,10). Unsaturated free fatty acids have been observed to be more effective than their saturated counterparts as absorption enhancing agents (3,5). A review of the literature reveals little recent data on the effects of lipids and surface active agents on the epithelia of the lung, despite the fact that they are often administered as components of aerosol formulations. In addition, as far back as 1928, Pinkerton (11) introduced a series of saturated and unsaturated oils to the airways of dogs and rabbits and, in some instances, noted severe damage to the lung which he concluded was due to free fatty acids (FFAs). Later, in the 1950's, Peltier (12), who was studying the nature of pulmonary embolism, demonstrated that unsaturated fatty acids introduced via the circulation caused pulmonary pneumopathy which was markedly different from that produced by saturated fatty acids and that mechanical obstruction of the pulmonary vascular bed was not the sole reason for the resultant edema. A number of studies have been carried out to determine the mechanism of action of oleic acid and other free fatty acids when introduced intravenously to the pulmonary circulation (13–20), and a dose–response relationship has been noted for the quantity of oleic acid administered and the degree of edema produced (14,16). The pronounced lung toxicity does not appear to apply to the saturated fatty acids such as palmitic acid or neutral fats (11,12,23). Since a number of metered dose inhaler products contain either Span 85 or oleic acid as dispersing and lubricating agents, they therefore have the potential of damaging the lung tissue and altering its permeability to the administered drug. Also, there recently have been a number of proposals to deliver anticancer and antimicrobial agents to the lung encapsulated in lipid vesicles (24–26). Encapsulation efficiencies are often low, depending upon what type of vesicles are used (27), and so the quantities of lipid which might be introduced to the lung could be large relative to the drug. Given that most liposomes are based upon phosphatidylcholine, which contains significant quantities of unsaturated fatty acids (28), and that lung esterases are capable of hydrolyzing the diesters (29), then there is the possibility that significant levels of unsaturated fatty acids may be released in the peripheral

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lung. Indirect evidence for this may be drawn from a publication by Lindahl *et al.* (30), where they show that lysophosphatidylcholine produces increased permeability of the lung to polyethylene glycol. The increased permeability could have been due partially to the release of unsaturated fatty acids from the phospholipid particularly as large doses were being administered.

As far as is known no concerted effort has been undertaken to quantify the effects of oleic acid and other surfactants when introduced to the airways. In view of the use of surfactants in MDIs and the potential that lipids may be introduced to the airways with encapsulated drugs, this paper describes the independent effects of oleic acid, oleyl alcohol, and Span 85 on the epithelial permeability of the isolated perfused rat lung (IPRL) to the marker dye fluorescein. It is hoped that the results provide perspective on the mechanism of altered permeability and that surfactant levels which can be inhaled "safely" by humans can be estimated.

MATERIALS AND METHODS

The isolated perfused rat lung (IPRL) preparation which was employed in these investigations has been described in detail elsewhere (32,33). In the present study, individual IPRL experiments were replicated, with $n \geq 6$ in all cases. Experiments were designed to compare fluorescein absorption from aqueous solution (transfer from the airways to the perfusate of the IPRL) in the presence and absence of previously administered surface active agents. In all cases, the dye was administered as 100 μl of a 1 mg/ml solution of disodium fluorescein using the "cartridge" technique described previously (32). Briefly, the technique involved the expulsion of the aqueous solution into the airways as a coarse spray, from an intratracheal dosing cartridge, while simultaneously inflating the lungs with a fixed volume of fluorocarbon gas. The method results in the delivery of a reproducible amount of dye to the peripheral airways, from which absorption subsequently occurs. Lipophilic surface active agents were administered 90–105 seconds prior to the dye from MDIs containing different surfactant concentrations dissolved in fluorocarbon propellants. Control experiments involved fluorescein transfer in the absence of surfactants but utilized a sham procedure whereby propellants alone were administered by MDI, 90–105 seconds prior to dosing with fluorescein. The surfactant formulations and the exact dosing methodology are described in more detail below.

Surfactant Preparations

The surfactants studied were oleic acid, oleyl alcohol (Fisher Scientific, Springfield, NJ), and Span 85 (Fluka Chemical Corp., Ronkonkoma, NY). All are liquids at 37°C. The acid and alcohol contain a single double bond placed centrally in their C-18 hydrocarbon chain. Span 85 is a mixture of mono-, di-, and tri-esters of sorbitan with long-chain fatty acids which consist chiefly of oleic acid. Surfactants were preweighed into 15-ml plastic-coated glass aerosol containers (Wheaton Glass, Mays Landing, NJ). The vials were then pressure filled through the valve (Pamasol, Willi Mäder AG, Pfäffikon, Switzerland) with 10 g of a chlorofluorocarbon blend (1:2:1; Dymel-11:Dymel-12:Dymel-114, E. I. Dupont de Nemours & Co. Inc., Wilmington, DE), producing final concentrations of surfactant in solution ranging from 0 to 5% (w/w) (Table I). A 25-μl Valois DF30 valve assembly (BLM Packaging Inc., Greenwich, CT) was crimped on all containers, which were finally stored in the dark at 22°C. Oleic acid was prepared in surfactant concentrations of 5, 1, 0.5, and 0.1% (w/w). Oleyl alcohol and sorbitan trioleate were examined at 5 and 0.1% (w/w).

Surfactant Dosing to the Lung

Surfactant-containing MDIs were fitted with a modified Valois 251 EB/407/75-mm actuator (BLM Packaging Inc., Greenwich, CT). This actuator has an elongated (75-mm) tube as a spray nozzle, which was removed and replaced with one constructed of 316 stainless steel measuring 105 mm in length and having internal and external diameters of 0.89 and 1.64 mm, respectively. During surfactant dosing to the IPRL, MDIs were fitted with this actuator and inserted inside the tracheal cannnulae (32,33) so that the end of the actuator tube projected to the end of the tracheal cannulae inside the biological trachea. During actuation, a rapidly evaporating divergent spray of the surfactant formulation was expelled into the biological trachea, which also inflated the lungs with ≈6 ml of fluorocarbon vapor (32,33). Since absorption from this preparation can take place only from the peripheral regions of the lung containing intact vascula-

Table I. Surfactant Preparations Used to Dose the Isolated Lung

Surfactant	Nominal % (w/w)	Surfactant weight (mg)	Propellant weight (g)	Final % (w/w)[a]	Dose/actuation (dose to lung)[b]
Oleic acid	5.0	503.4	10.05	4.77	1660 (199)
	2.5	252.4	10.04	2.45	853 (102)
	1.0	98.9	9.67	1.01	352 (42)
	0.1	11.3	10.06	0.11	39 (5)
Oleyl alcohol	5.0	503.6	9.77	4.90	1706 (205)
	0.1	12.3	9.85	0.12	43 (5)
Span 85	5.0	498.1	9.89	4.79	1668 (200)
	0.1	12.8	9.94	0.13	45 (5)

[a] Final percentage (w/w) of surfactant contained in the MDI.

[b] Estimated dose of surfactant leaving the MDI upon actuation, in micrograms and, in parentheses, the estimated dose of surfactant, in micrograms, reaching the lung lobes.

ture (34,35), the dose of surfactant penetrating these regions required estimation.

The amount of surfactant leaving the actuator per dose was determined by preparing and packaging a formulation containing 0.01% (w/w) fluorescein solubilized in propellants by 5% (w/w) Span 85 (36) and actuating it into a 50-ml collection vessel followed by a fluorometric assay of the fluorescein (31). The dose of surfactant leaving the actuator was determined from

$$\text{surfactant dose} = \frac{\text{amount of fluorescein assayed}}{\text{fluorescein concentration in propellants}} \times \text{surfactant concentration in propellants} \quad (1)$$

The gross distribution of dose reaching the lung was determined by preparing isolated lungs as described previously (32) and dosing them five times with this formulation using the modified actuator described above. After each dose the lungs were allowed to deflate to 1- to 2-ml residual volume before the next actuation. The total procedure took no longer than 30 sec. Perfusion was then immediately halted and the lungs were removed from the artificial thorax (32). The right lung (RL), left (LL), and trachea (TR) were separated and each assayed for their content of fluorescein. Likewise the tracheal cannula (T) and tracheal exit cannula (TE) were also analyzed for the presence of the dye (32). This process was repeated for eight individual isolated lung experiments.

Transfer Kinetics of Fluorescein

The effects of surfactants on the permeability of fluorescein were assessed by the changes in rates of absorption according to a first-order model

$$F_a = F_{a_{inf}} * [1 - e^{(-k_a * t)}] \quad (2)$$

where F_a is the amount of fluorescein absorbed as a function of time, t; $F_{a_{inf}}$ is the amount which would be absorbed at infinity; and k_a is the first-order rate constant for absorption. The half-lives of absorption, $t½$, were then obtained from $(\ln 2)/k_a$ and compared for each surfactant against control results, where no surfactant was employed in the MDIs, using Student's t test for paired samples. An analysis of variance (ANOVA) was also performed on the oleic acid results.

RESULTS AND DISCUSSION

Surfactant Dosing to the Lung

The fluorescein output leaving the actuator from the formulation containing 0.01% (w/w) fluorescein was 2.08 ± 0.17 μg (n = 8). Consequently to determine the distribution of dose to isolated lungs, the MDI was actuated five times to ensure that sufficient fluorescein would be present to assay accurately. The percentage of recovered fluorescein reaching the lung lobes (RL + LL) was small, 12.1 ± 4.7%, but quite reproducible (Fig. 1). The relatively poor efficiency was due to divergence of the spray, resulting in the majority of the recovered dose impacting directly on the walls of the trachea, 53.2 ± 3.6%. A significant component was also flushed into the tracheal cannulae, 33.1 ± 6.1% (Fig. 1), due to the back pressure in the airways immediately after actuation. The results clearly support the fact that, close to the spray nozzle, propellant droplets are large and have large impaction efficiencies (22). In fact, it is unlikely that complete evaporation of fluorocarbons has taken place before droplets reach the peripheral lung, as the distance between the nozzle and the deep lung was short, no greater than 5 cm. Evidence of this is suggested from work by Byron *et al.*, (37) who show that of the total dye aerosolized from pressure packs, the percentage deposition within 5-cm-length mouthpieces is apparently unaffected by the concentration of surfactant used, which implies that the droplets had similar size and size distributions (37). If so, then for the surfactants and their tested concentrations, an estimated 12% of the recovered dose leaving the actuator can be presumed to reach the lung periphery, where they may or may not influence the absorption kinetics of fluorescein administered some 100 seconds later.

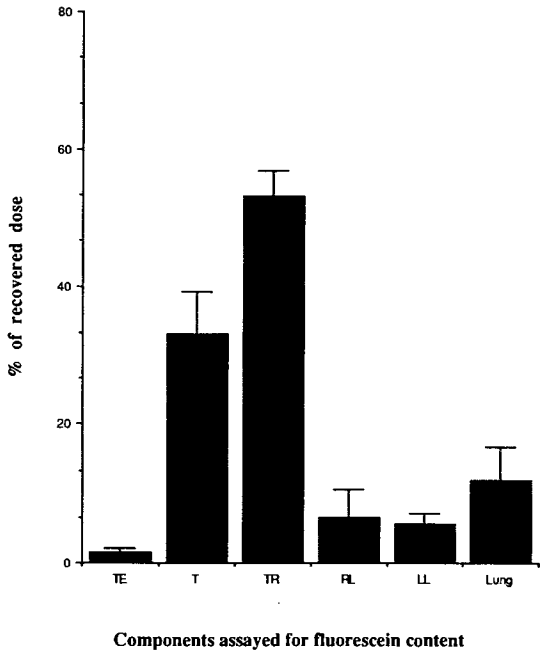

Fig. 1. The distribution of solubilized fluorescein in the tissue and nontissue components of the isolated lung after direct delivery from a metered dose inhaler. The percentage recovered dose found in each region was 1.6 ± 0.4 in the tracheal exit cannula (TE), 33.1 ± 6.1 in the tracheal cannula (T), 53.2 ± 3.6 in the rat trachea (TR), 6.5 ± 4.1 in the right lung (RL), 5.6 ± 1.6 in the left lung (LL), and thus 12.1 ± 4.7 in the left and right lung (lung). This indicates that ≈12% of a dose of surfactant leaving the actuator will deposit in the peripheral lung.

Surfactants and the IPRL

Oleic acid delivered from the pressure packs containing 5, 2.5 and 1% (w/w) quantities all significantly affected the absorption of fluorescein relative to controls. The dose delivered from the 0.1% (w/w) container did not alter the absorption of the dye (Fig. 2). A dose–"response" relationship was seen to exist between the degree of fluorescein absorp-

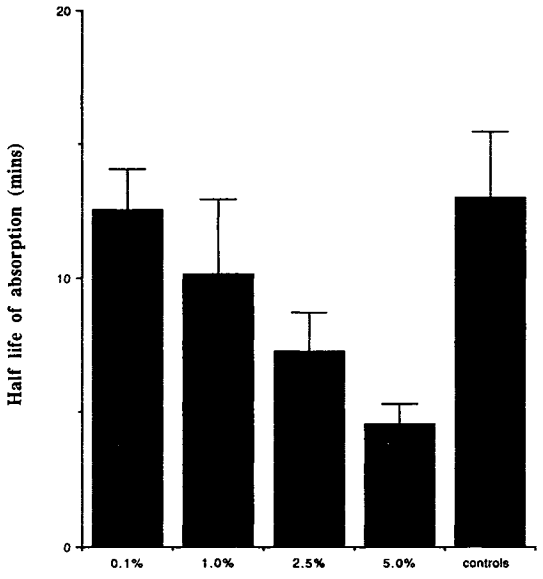

Fig. 2. The effect of oleic acid on the absorption of fluorescein. The acid was delivered to the airways from a MDI fitted with a 25-μl valve and containing 0 (controls), 0.1 (4 μg), 2.5 (100 μg), and 5.0% (w/w) (200 μg) of the surfactant. The values in parentheses are the quantities which would be expected to reach the lung lobes. The half-lives in minutes ± standard deviations for six experiments conducted with each MDI were 12.5 ± 1.5 (0.1% w/w), 10.2 ± 2.8 (1.0%, w/w), 7.5 ± 1.5 (2.5%, w/w), and 4.5 ± 0.8 (5.0%, w/w). Control results were 12.9 ± 2.5 min. ANOVA showed that the degree of absorption enhancement of fluorescein was dependent upon the dose of oleic acid (F = 917.3 with 3 and 20 df).

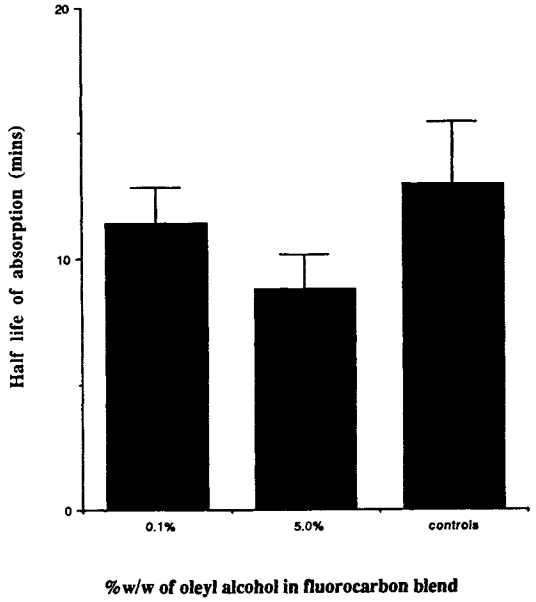

Fig. 3. The effect of oleyl alcohol on the absorption of fluorescein. The alcohol was delivered to the airways from a MDI fitted with a 25-μl valve and containing 0 (controls), 0.1 (4 μg), and 5.0% (w/w) (200 μg) of the surfactant. The values in parentheses are the quantities which would be expected to reach the lung lobes. The half-lives in minutes ± standard deviations for six experiments conducted with each MDI were 11.4 ± 1.4 (0.1%, w/w) and 8.6 ± 1.4 (5.0%, w/w). Control results were 12.9 ± 2.5 min.

tion enhancement and the quantity of oleic acid introduced to the deep airways. At the highest dose, equivalent to an estimated 200 μg reaching the alveolar and intrapulmonary bronchiolar regions, absorption half-lives of fluorescein were reduced from 12.9 ± 2.5 min for controls to 4.5 ± 0.8 min and all lungs became grossly edematous within 40 min of dosing the surfactant. Visible changes in the lung parenchyma were noticeable within minutes. Therefore the absorption enhancement of fluorescein by oleic acid was associated with some toxic and damaging effect on the pulmonary barrier including the alveolar epithelium, which is assumed to be the rate-limiting barrier to transfer of compounds from the airways to the circulation.

Oleyl alcohol also showed increased absorption of fluorescein (Fig. 3) but not to the same extent as the corresponding acid. The carboxylic acid group thus affects the membrane permeability more profoundly than the somewhat less polar aliphatic hydroxyl group. This observation is consistent with substantial evidence (1,2,38—41) that the permeability enhancement is due to both the polar head group and the hydrocarbon chain. Local acidity produced by ionization of the fatty acid might also contribute to membrane damage but such an effect is limited by the low aqueous solubility of oleic acid. Hirsch (42) suggests that the lowest pH possible in an aqueous medium is about 5.7.

Span 85 also enhanced the absorption of the fluorescein to a similar extent to that of oleyl alcohol (Fig. 4). The effect produced by the surfactant in this case is probably due to a specific action of Span 85 as a surfactant. Hydrolysis of the sorbitan oleyl esters by esterases in the lung (29) releasing oleic acid is another possibility, but given the relatively short experimental time frame it is unlikely that sufficient accumulation of oleic acid could take place to produce the observed effects.

It is unlikely that the doses of surfactant contained in marketed inhaler products are capable of causing significant short-term toxicity or of altering the absorption of the inhaled drug. The equivalent dose which a human patient might expect to receive in order to produce similar effects would be enormous. The average lung surface area of a 300-g rat is about 0.4 m^2 (43), whereas that of a 70-kg male is about 75 m^2 (43). Using the surface area to provide a scaling factor between rat and human, it can be estimated that to observe severe damage in the human lung (75/0.4) × 200 μg = 37,500 μg or some 0.375 g oleic acid would need to be administered. A dose of 4 μg (0.1%, w/w), on the other hand, which did not produce changes in fluorescein transfer in the rat, is equivalent to 7500 μg in the human. The highest single inhaled dose of drug from any product containing oleic acid is ≈100 μg, of which only about 10% would be expected to deposit in the lung (44). It seems unlikely that a single inhalation from a marketed inhaler containing the fatty acid is capable of inducing changes in pulmonary barrier permeability.

It is clear that all the surfactants tested are capable of producing increased absorption of fluorescein, but it is still not clear exactly what changes and how many are taking place in the pulmonary barrier. There are a number of possibilities. Evidence of a membrane perturbing action is

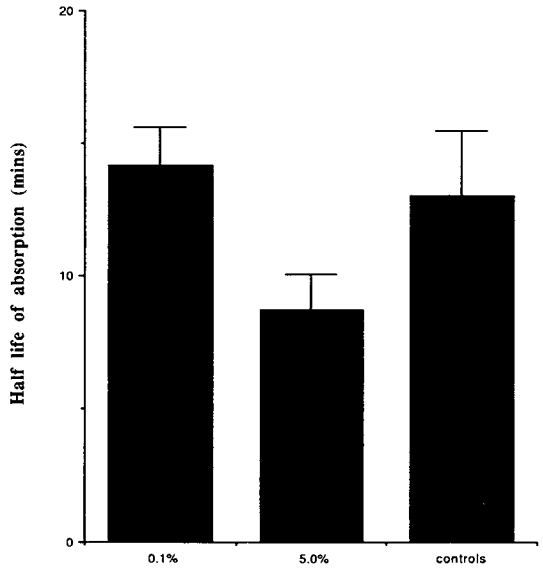

Fig. 4. The effect of Span 85 on the absorption of fluorescein. The Span 85 was delivered to the airways from a MDI fitted with a 25-μl valve and containing 0 (controls), 0.1 (4 μg), and 5.0% (w/w) (200 μg) of the surfactant. The values in parentheses are the quantities which would be expected to reach the lung lobes. The half-lives in minutes ± standard deviations for six experiments conducted with each MDI were 14.2 ± 1.4 (0.1%, w/w) and 8.7 ± 1.4 (5.0%, w/w). Controls were 12.9 ± 2.5 min.

strong and this may be the primary effect of the fatty acids (38–41). This is also consistent with a dose–response relationship since the "leakiness" of the membranes might be coincident with the quantity of incorporated fatty acid in the membrane bilayers. With reference to the deposition of surfactants on the surface of the lung, complete disruption of the surfactant lining layer may take place, causing increased local surface tension, which could produce either collapse of the alveoli or edema through altered transpulmonary pressures (45). However, Evander *et al.* (46) showed recently that although dioctyl sodium sulfosuccinate accelerated the clearance of ^{99m}Tc-DTPA from the lungs of rabbits, no significant changes were noted in the alveolar surface tension. Local acidity is a further possibility with the fatty acids (42), and although saturated fatty acids do not appear to be as toxic as unsaturated ones, the former have not been introduced to the airways directly to test this idea. Some interaction between the fluorescein and the surfactants in the airways might also contribute to the results, but given the low aqueous solubility of the oleic acid and the presence of a complex natural surfactant system in the lung, it is difficult to speculate on this subject. Complexation might be expected to retard the absorption of fluorescein but, on the other hand, could enhance its absorption by increased lipophilic character of a solute-surfactant complex.

While the lung is a possible port of entry for poorly absorbed compounds with systemic activity, consideration should be given to screening absorption enhancement in a simple experimental lung model such as that described here. The ease with which the IPRL may be used as a means of studying enhancement and/or chemically mediated membrane toxicity is demonstrated clearly in Figs. 2, 3, and 4. The possibility that lipid vesicles and other novel surface active materials can be used as pulmonary drug delivery systems (24–26), where the quantity of these excipients may exceed the drug per unit dose, make it pertinent to study potential toxicity of the individual lipids as well as the drug itself.

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