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Surfactant as a drug carrier

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ABSTRACT

Drug delivery using a surfactant vehicle has the potential to prevent systemic side effects by delivering therapeutic agents directly to the respiratory system. The inherent chemical properties of surfactant allows it to readily distribute throughout the respiratory system. Therapeutic agents delivered by surfactant can primarily confer additional benefits but have potential to improve surfactant function. It is critically important that additional agents do not interefere with the innate surface tension lowering function of surfactant. Systemic evaluation through benchtop, translational and human trials are required to translate this potential technique into clinical practice.

Practice points

- Local delivery of therapeutic agents to the respiratory system may mitigate side-effects associated with systemic administration
- Surfactant may be a suitable vehicle for delivery of therapeutic agents throughout the respiratory system
- Additional therapeutic agents should not interfere with the innate surface tension lowering function of surfactant
- Development of drugs delivery by surfactant vehicles requires systematic evaluation through preclinical and clinical trials

1. Introduction

Medical practitioners constantly grapple with the challenge of mitigating the side effects associated with systemically administered therapies. The seemingly logical alternative would be the targeted delivery of medications to specific organs, which has the potential to transform therapeutic drug administration. A prominent example of organ specific, targeted drug application in neonatal medicine is the administration of pulmonary surfactant [1]. Pulmonary surfactants are a naturally occurring intricate mixture of phospholipids, cholesterols and proteins which play an important role in lowering surface-tension at the pulmonary air-liquid interface, and modulating host immune responses [2]. Surfactant replacement therapy with synthetic or natural animal derived surfactants (exogenous surfactants) marked a turning point in neonatology and contributed significantly to the increased survival of premature infants, and is the recommended therapeutic approach for newborn preterm infants with respiratory distress syndrome (RDS) [3–6]. Exogenous surfactant that is administered directly into the trachea by means of a tube or catheter inserted through the vocal cords readily distributes to the peripheries of the pulmonary tree. This renders exogenous surfactant a potentially powerful 'vehicle' to transport therapeutic agents to and throughout the lungs. The direct application of pharmacological agents to the target organ may minimize side effects associated with systemic administration of those agents. While using exogenous surfactant as a vehicle for other drugs may confer additional therapeutic benefits, it is important that those drugs are well distributed through the respiratory system, and the admixing does not interfere with the innate surface activity of exogenous surfactant.

This chapter will examine the application of exogenous surfactant as a drug carrier (surfactant vehicle) in newborn infants, explore the underlying mechanisms by which surfactant facilitates drug distribution within the respiratory system, the essential characteristics of surfactant additives that make them amenable to this, and the variety of drugs that may be delivered using a surfactant vehicle. Furthermore, we will explore agents that either augment surfactant function or provide independent therapeutic benefits. The second part of this chapter is

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Abbreviations

ARDS	Acute respiratory distress syndrome
BPD	Bronchopulmonary dysplasia
CAT	Catalase
FiO_2	Fraction of inspired oxygen
IL	Interleukin
MAP	Mean airway pressure
MAS	Meconium aspirations syndrome
PEG	Polyethylene glycol
RDS	Respiratory distress syndrome
SOD	Superoxide dismutase
VLBW	Very low birth weight

dedicated to the administration of corticosteroids using a surfactant vehicle, as this topic contributes to a significant portion of the existing literature pertaining to the utilization of surfactants as drug carriers and is a 'hot topic' in the field. Finally, we will address potential future directions for the use of surfactant vehicles in newborn medicine and outline the challenges that must be surmounted to successfully integrate this innovative approach into clinical practice.

2. Rationale for surfactant as a drug carrier

2.1. Surfactants have unique biochemical properties

In the years following Avery and Mead's revolutionary discovery of surfactant deficiency in preterm infants in 1959 [7], researchers isolated and characterize individual components of surfactant and demonstrated how they contributed to surfactant function [8]. Since then, clinical trials have demonstrated a significant reduction in mortality and morbidity in preterm infants with respiratory distress syndrome treated with exogenous surfactants [3,9]. Surfactant is an amphiphilic complex mixture of phospholipid and proteins, possessing both hydrophobic and hydrophilic regions within its molecular structure [10]. When incorporated with other biologically active agents, surfactant solubilizes and stabilizes additive compounds within a solution [11]. As a result, this may enhance the absorption and bioavailability of therapeutic agents by increasing solubility and permeability across cell membranes.

2.2. Surfactant extensively distributes through the respiratory system

The unique surface active properties of surfactant facilitates ready distribution and adsorption through the respiratory system after tracheal instillation [12]. The amphiphilic molecular structure allows hydrophobic regions to interact with both the air within the alveoli while the hydrophilic regions simultaneously interact with the liquid lined alveolar membranes. Furthermore, the tracheal instillation of surfactant induces a surface tension gradient, resulting in molecules within areas of lower surface tension moving towards areas of high surface tension. Coined the 'Marangoni effect' after its first description in 1865 [13], this fluid dynamics phenomenon results in net flow of liquid towards areas of high surface tension and further enhances distribution of surfactant into the lung peripheries (Fig. 1). In combination, these properties make exogenous surfactants a well-equipped vehicle for drug delivery to the respiratory system.

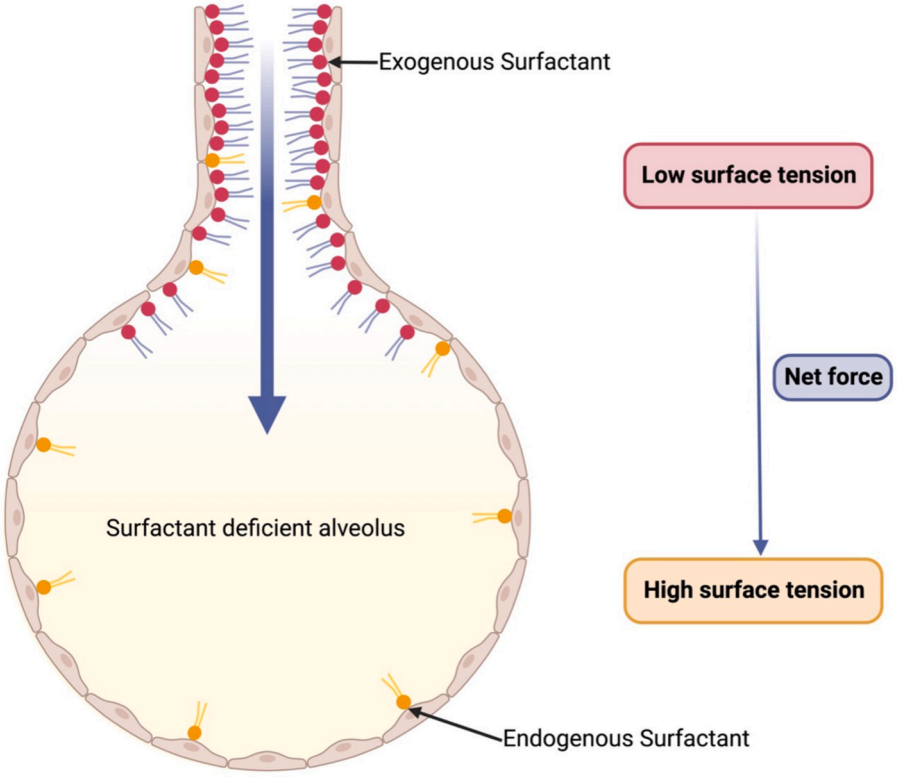

Fig. 1. Illustration of the Marangoni effect. A surface tension gradient (large blue arrow) is induced due to the relative difference in surfactant concentration between exogenous (red) and endogenous (orange) surfactant. The net positive force directed towards the alveolus results in extensive distribution of surfactant to the lung peripheries. "Created with BioRender.com".

Table 1
Studies investigating the impact of addition of non-corticosteroid drugs on innate surfactant function.

Author (year)	Additive	Surfactant type	Method/Model	Results	Comments
Banerjee (2001)	Calcium chloride	Synthetic (pure DPPC, PE and PG)	Wilhelmy plate, bubble surfactometer and examination of ultrastructure using electron microscopy	5 mM of calcium decreased compressibility, increased liposome stability and formed microtubule structures in all surfactant preparations	No further benefit was found by adding 20 mM of calcium to surfactant preparations. Pure DPPC, PE and PG not used in clinical practice
Banerjee (2001)	Eucalyptus oil	Survanta ALEC Exosurf DPPC, PE and PG	Wilhelmy plate, bubble surfactometer and examination of ultrastructure using electron microscopy	Addition of eucalyptus oil improved adsorption of all surfactant preparations but reduced stability of all surfactants except DPPC and PG	Safety of intratracheal eucalyptus oil not established
Huang (2004)[a]	Lidocaine	Bovine surfactant Infasurf	Bubble surfactometer	Surface activity was not altered by addition of lidocaine	Also examined impact of surfactant + lidocaine vs surfactant on lung function
Calkovska (2007)	Dextran	Porcine Curosurf	Newborn rabbit albumin induced RDS model	Dextran improved therapeutic effects of low dose (40 mg/kg) but not high dose surfactant (200 mg/kg).	Investigators postulated that Dextran increased viscosity of high dose surfactants, impairing distribution after instillation.
Bronshtein (2009)	Vitamin A (derivatives)	Bovine surfactant BLES	Bubble surfactometer, and microscopic and macroscopic visualization for miscibility and solubility	Retinal acetate admixtures were associated with better miscibility and solubility but reduced surface activity by 40%	Modern surfactant preparations not tested
Lyra (2009)	PEG	Porcine Surfactant Butantant	Newborn rabbit meconium aspiration model	Addition of PEG to surfactant did not improve lung compliance, lung volumes or alveolar aeration	Outcomes measured after a short 20 min period of mechanical ventilation
Sing (2010)	Vitamin A (retinol acetate)	Bovine surfactant BLES	Newborn piglet RDS model	Addition of retinol acetate to surfactant did not alter efficacy of surfactant	Retinol acetate was systemically bioavailable after intratracheal administration

DPPC; dipalmitoyl phosphatidylcholine, mM; millimolar, PE; phosphatidylethanolamine, PEEP; positive end expiratory pressure, PEG; polyethelene glycol, PG; phosphatidylglycerol, RDS; respiratory distress syndrome.
[a] Refer to Table 2 for further details on study.

3. Key features of surfactant additives

3.1. Drugs added to surfactant should not impair innate surfactant function

Pulmonary surfactants serve a critical role in maintaining newly gained lung volume and gas exchange after birth. Through active reduction of surface tension, pulmonary surfactants prevent alveolar collapse at the end of expiration and facilitate continuous gas exchange. It is therefore critical that any added therapeutic agent does not disrupt the inherent surface activity of surfactant itself. Accordingly, it becomes imperative to conduct in-vitro experiments aimed at evaluating the surface activity of surfactants before and after their combination with supplementary therapeutic agents.

Surfactant functions can be measured in several ways. The Wilhelmy plate or Wilhelmy balance method involves suspending a thin metal plate, usually made of platinum, nickel or glass, at the air-liquid interface of the solution under investigation. The force required to remove the plate from the interface is then measured using a tensiometer or microbalance to calculate the surface tension of the solution [14]. When used in combination with a Langmuir balance, surfactant compressibility (which is inversely proportional to surface tension) can also be measured [15]. A bubble surfactometer works by generating a single bubble by injecting gas (usually air) into the surfactant solution. By pulsating the bubble size and monitoring the subsequent changes in the resulting pressure-gradient, the surface tension at the air-liquid interface can be calculated [16]. The miscibility (the ability of two liquid solutes to mix and form a homogenous solution) and solubility (the ability of a solute to dissolve into a liquid without precipitating) can be assessed with macroscopic and microscopic visual examination. Examination of surfactant microstructure has been performed using electron microscopy [17,18] and atomic force microscopy [19,20].

Numerous therapeutic agents have been evaluated with these techniques. The addition of low concentration ionized calcium has been shown to improve chemical stability in multiple synthetic surfactant preparations [17]. Eucalyptus oil, which was previously used as an expectorant in chronic bronchitis, improves adsorption of synthetic surfactant at the cost of reduced stability [18]. Retinol (Vitamin A) derivatives have been found to reduce surface activity of surfactant in-vitro [21] however this was not replicated in-vivo in neonatal piglet models of RDS [22]. Details of these studies are shown in Table 1.

3.2. Supplementary drugs may improve innate surfactant function

Surfactant inactivation has been implicated as a significant pathological factor in numerous neonatal respiratory disorders including meconium aspiration syndrome (MAS), pulmonary haemorrhage and acute respiratory distress syndrome (ARDS) secondary to systemic inflammation. Consequently a small number of studies have studied the use of supplementary agents to augment innate surfactant function. Lyra et al. (2009) evaluated the potential benefits of incorporating polyethylene glycol (PEG) to surfactant in a newborn rabbit model of MAS [23]. Disappointingly PEG did not yield significant improvement in lung mechanics or histopathological outcomes compared to surfactant alone. Dextran, a non-ionic polymer based plasma substitute, has been demonstrated to improve surfactant treatment effect following a suboptimal dose (40 mg/kg of Curosurf) in newborn rabbit model of albumin induced RDS, but this benefit was not reproduced when using therapeutic doses (200 mg/kg of Curosurf) [24].

Despite the inconclusive findings of these preliminary studies, augmentation of inherent surfactant function remains a compelling avenue of research. Details of these studies are shown in Table 1.

3.3. Therapeutic benefits and improved delivery of additional therapeutic agents using surfactant as a vehicle

The primary objective of employing surfactant vehicles is to facilitate the delivery of therapeutic benefits conferred by the secondary agents. Additionally, using surfactant as a vehicle may result in better drug distribution due to the unique chemical properties that have been

Table 2
Non-corticosteroid drugs conferring additional benefits or resulting in improved delivery after admixing with surfactant.

Author (year)	Model	Drug	Outcome	Results	Comments
Kharasch (1991)	Hamsters (n = 10)	Pentamidine (radio-labelled)	Distribution of pentamidine using either surfactant or saline as a vehicle	Delivery with surfactant resulted in better distribution of pentamidine	All hamsters were healthy and spontaneously breathing
Huang (2004)	Neonatal piglets (n = 10) HCl induced ARDS	Lidocaine	Gas exchange, lung mechanics and histopathology	Improved PaO_2 and $PaCO_2$ seen in groups treated with surfactant + lidocaine compared to surfactant alone. No difference observed in lung compliance or histopathology	Surfactant given using a dilute lavage
Obaid (2005)	Neonatal piglets (n = 12) Thromboxane A2 induced PHTN	Sildenafil	Pulmonary arterial pressure and pulmonary vascular resistance	Sildenafil + surfactant resulted in greater improvement in PAP and PVR	
Dani (2014)	Preterm lambs (n = 14)	SOD + CAT	Lung tissue oxidative stress	Markers of oxidative stress (TH, AOPP and NPBI) did not increased in surfactant + SOD + CAT groups but did in surfactant only group	Utilized an injurious ventilation strategy
Basabe-Burgos (2019)	Near term rabbits (n = 40)	Polymyxin E	CFU count in lung homogenate post mortem	Surfactant and polymyxin resulted in the greatest reduction in CFU in vivo, despite reduced antibacterial activity in-vitro	

AOPP; advanced oxidation protein products ARDS; acute respiratory distress syndrome, CFU; colony forming unit, HCl; hydrochloric acid, NPBI; non-protein bound iron, PAP; pulmonary arterial pressure, PHTN; pulmonary hypertension, PVR; pulmonary vascular resistance, TH; total hydroperoxide.

described earlier. The addition of corticosteroids constitutes a substantial portion of the current literature and will be elaborated upon separately. Other agents are currently being explored in preclinical studies which utilize various animal models of respiratory distress. Huang et al. (2004) demonstrated that the addition of lidocaine to surfactant during surfactant lavage in a hydrochloric acid-induced ARDS model in neonatal piglets [25] resulted in improved lung mechanics and gas exchange when compared to lavage with surfactant alone. In a neonatal piglet model of thromboxane A2 analogue induced pulmonary hypertension, Obaid et al. (2005) demonstrated benefits of adding sildenafil to surfactant in reducing pulmonary arterial pressure and pulmonary vascular resistance [26]. Similarly, the addition of superoxide dismutase (SOD) and catalase (CAT) to surfactant resulted in reduced markers of oxidative stress in mechanically ventilated preterm lambs [27]. Kharasch et al. (1991) demonstrated effective delivery of radiolabeled pentamidine in healthy hamsters, highlighting again the ability of surfactant to aid in drug delivery through the respiratory system [28]. Interestingly, surfactant-drug combinations may exhibit different effects depending on in-vitro or in-vivo environments. Polymyxin E, an anti-pseudomonal antibiotic, exhibits reduced anti-bacterial activity in-vitro but increased activity in-vivo, potentially due to better distribution with a surfactant vehicle [29]. These experiments highlight advantages of incorporating therapeutic agents with surfactants, demonstrating not only the supplementary benefits provided but also the improved drug delivery facilitated by surfactant vehicles. Details of these studies are shown in Table 2.

3.4. *Using surfactant as a vehicle to administer intrapulmonary corticosteroids*

Due to the association with adverse neurodevelopmental outcomes, use of systemic corticosteroids for the management of severe lung disease is reserved for the most unwell, respiratory challenged preterm infants. As corticosteroids are well dissolved into pulmonary surfactants

Table 3
Studies investigating the impact of addition of corticosteroids on innate surfactant function.

Author (year)	Corticosteroid	Surfactant type	Ratio[a]	Method/Model	Results	Comments
Nimmo (2002)[b]	Dexamethasone	Bovine (Surfanta)	250:1	Bubble surfactometer	Surface activity of Survanta was not altered by the addition of dexamethasone	Also examined distribution of dexamethasone with surfactant as a carrier vs. normal saline.
Wang (2012)	Budesonide Beclomethasone	Bovine (Infasurf)	100:1 budesonide 10:1 Beclomethasone	Langmuir Balance Atomic force microscopy	Concentrations of 1 % Budesonide and 10 % Beclomethasone did not alter surface activity of surfactant	Concentrations beyond this resulted in early collapse of surfactant films due to fluidization
Zhang (2012)	Budesonide	Porcine (Curosurf)	10:1	Langmuir balance Atomic force microscopy	Concentrations of up to 10 % budesonide did not alter surface activity of surfactant	Also tested pure cholesterol. 10 % pure cholesterol completely inactivated surfactant
Cimato (2016)	Budesonide Beclomethasone Fluticasone	Bovine (Prosurf)	10:1	ESPR Bubble surfactometer	10 % concentrations of each corticosteroid did not alter surface activity of surfactant	
Palmer (2000)	Budesonide	Bovine (Survanta, BLES)	"Low": 16:1 "High": 0.5:1	Bubble surfactometer	Both budesonide surfactant concentrations impaired surfactant function	
Chen C (2019)[b]	Budesonide	Porcine (Curosurf) Bovine (Survanta)	Curosurf: 160:1 Survanta 50:1	Bubble surfactometer Drop shape tensiometer HPLC	At the set concentrations, budesonide did not impair function of each surfactant preparation	Also tested surfactant distribution using radiolabeled budesonide

ESPR; Electronic spin resonance spectroscopy, HPLC; high performance liquid chromatography.
[a] Phospholipid:corticosteroid ratio.
[b] Refer to Table 5 for further details on study.

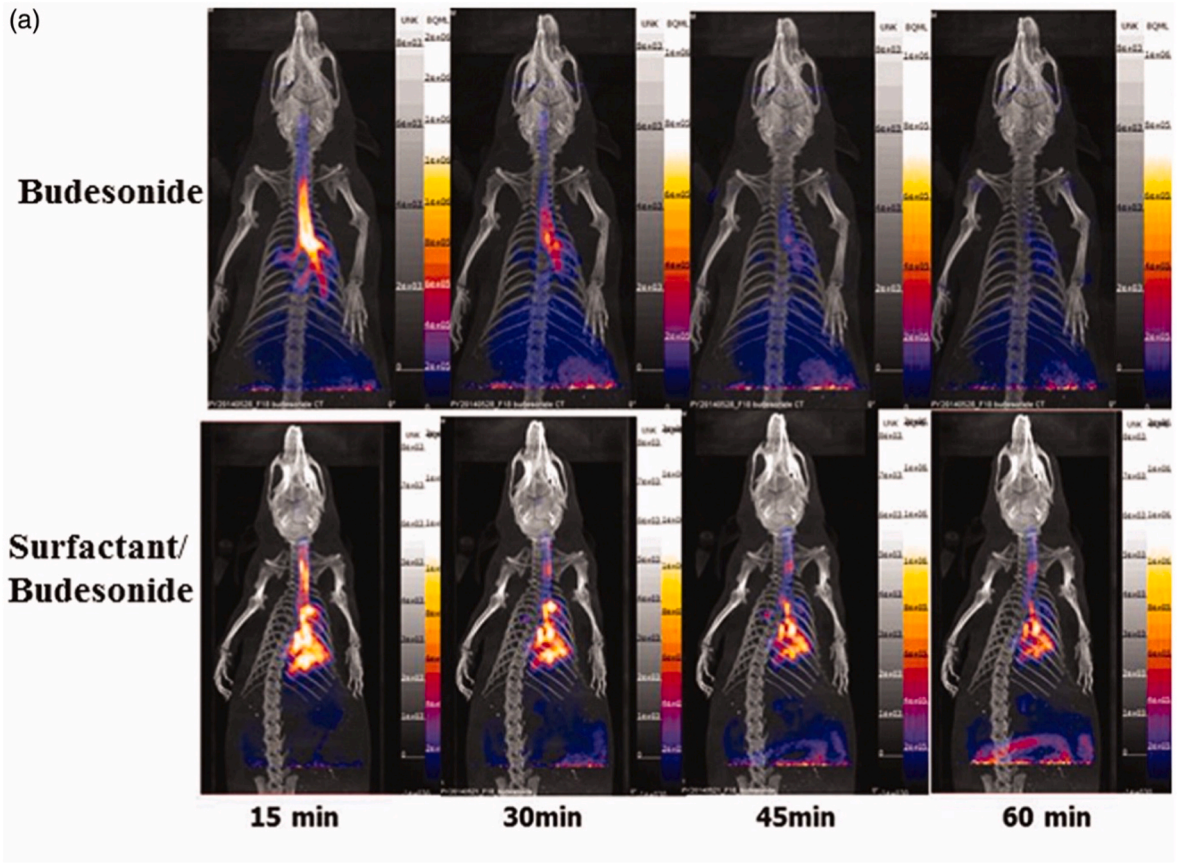

Fig. 2. Distribution of radiolabelled ^{18} F-Budesonide detected using Nano positron emission tomography digital scans in Sprague-Dawley rats after administration with and without a surfactant vehicle. Figure adapted from Chen et al. 2019 [37].

Table 4
Studies examining the pulmonary distribution and systemic absorption of corticosteroids administered via a surfactant vehicle.

Author (Year)	Model	Corticosteroid	Surfactant	Corticosteroid dose	Method	Results	Comments
Fajardo (1998)	Adult rabbits (n = 22)	Budesonide	Bovine (Survanta)	0.8–2.3 μg	Saline induced RDS (50 % population). Assessment of distribution of [^{3}H]Budesonide using autoradiography and liquid scintillation analysis	Budesonide was effectively distributed through the lungs using both saline and surfactant as a carrier	Effect of both surfactant and normal saline on budesonide distribution was similar
Nimmo (2002)	Adult rats (n = 18)	Dexamethasone	Bovine (Survanta)	40 μg/kg	Radio-labelled dexamethasone	Dexamethasone was more extensively distributed using a surfactant vehicle compared to a saline vehicle. Intratracheal administration resulted in a 10-fold reduction in peak drug concentration compared to IV administration	Also examined impact of dexamethasone in innate surfactant function
Huang (2015)	Adult mice (n = 30)	Budesonide	Bovine (Survanta)	0.25 mg/kg	Distribution of fluorescent dye after administration determined by fluorescence imaging	Combination of budesonide and Survanta results in the most extensive distribution of fluorescent dye	Mice were anaesthetized and not mechanically ventilated. Budesonide not specifically labelled
Roberts (2016)	Preterm lambs (n = 5)	Budesonide	Calfactant (Infasurf)	0.25 mg/kg	Intratracheal administration and plasma sampling. Post-mortem tissue analysis of brain and lung tissue.	Budesonide was detected in lung tissue but not in brain tissue post mortem.	
Chen (2019)	Adult rats (n = 6)	Budesonide	Porcine (Curosurf) Bovine (Survanta)	0.25 mg/kg	Distribution of radiolabeled budesonide	Surfactant resulted in higher and more extensive pulmonary distribution and concentration of budesonide compared to a saline vehicle.	

*Further study details in Table 4.

Table 5
Studies examining the impact of corticosteroids and surfactant on lung injury outcomes in animal models.

Author (Year)	Model	Corticosteroid	Surfactant	Corticosteroid dose	Outcome	Results	Comments
Dani (2009)	Preterm lambs (n = 18)	Beclomethasone	Porcine (Curosurf)	400 or 800 μg/kg	Oxidative stress and lung mechanics measured by TH, AOPP and NPBI	Reduced markers of oxidative stress in the lung and improved lung function	
Yang (2010)	Piglets (n = 15) Saline washout induced RDS	Budesonide	Bovine (Survanta)	0.5 mg/kg	Oxygenation and lung histology	Improved gas exchange, but the addition of budesonide did not confer any benefits	Negative finding for budesonide postulated to be due to short duration of experiment (4 h)
Dani (2011)	Preterm lambs (n = 18)	Beclomethasone	Porcine (Curosurf)	400 or 800 μg/kg	Lung inflammation measured by IL-8 and MIF levels in bronchial aspirates	Surfactant and budesonide reduced lung inflammation	
Yang (2013)	Piglets (n = 12) Saline washout induced RDS	Budesonide	Bovine (Survanta)	0.25 mg/kg	Oxygenation, markers of inflammation, histological injury	Improved oxygenation, reduced proinflammatory cytokines and resulted in less histological lung injury	Longer duration experiment (24 h)
Mikolka (2016)	Rabbit (n = 33) Meconium aspiration	Budesonide	Porcine (Curosurf; lavage)	0.25 mg/kg	Neutrophil counts on BAL, oxidative stress and cytokine mRNA expression	Budesonide and surfactant was most effective and reducing lung inflammation and oxidative damage	
Kothe (2018)	Preterm lambs (n = 38)	Budesonide	Porcine (Curosurf)	0.25 mg/kg and 1 mg/kg	Lung mechanics, inflammation measured by RT-PCR and immunohistochemistry, and histological injury	Increased lung maturation, better lung function and reduced injury	Placental support model used. Compared both CPAP only and injurious ventilation strategy
Kothe (2019)	Preterm lambs (n = 37)	Budesonide	Porcine (Curosurf)	0.25 mg/kg	Lung mechanics, inflammation measured by RT-PCR, BALF cytokine analysis, immunohistochemistry and histological injury	Improved lung mechanics, reduced inflammation and reduced lung injury	Lambs ventilated up to 24 h
Hillman (2020a)	Preterm lambs (n = 23)	Budesonide	Porcine (Curosurf)	0.25 mg/kg, 0.1 mg/kg or 0.04 mg/kg	Lung and systemic inflammation determined by RT-PCR and immunohistochemistry	Low dose budesonide (0.1 mg/kg and 0.04 mg/kg) were less effective at decreasing lung inflammation.	Surfactant preparations given prior to the onset of ventilation
Hillman (2020b)	Preterm lambs (n = 23)	Budesonide	Porcine (Curosurf)	0.25 mg/kg	Lung and systemic inflammation determined by RT-PCR and immunohistochemistry compared between normal and injurious ventilation strategies	Reduced lung inflammation in both normal and injurious ventilation groups.	Both injurious ventilation and budesonide resulted in altered gene expression in the liver and brain
Hillman (2021)	Preterm lambs (n = 16) Intra-amniotic LPS model	Budesonide	Porcine (Curosurf)	0.25 mg/kg	Lung and systemic inflammation determined by RT-PCR and immunohistochemistry	Reduced lung and systemic inflammatory response to LPS and ventilation.	Structural brain changes associated with LPS and ventilation were not reduced with budesonide + surfactant

AOPP; advanced oxidation protein products, BALF; bronchoalveolar lavage fluid, IL8; interleukin 8, LPS; lipopolysaccharide, MIF; macrophage migration inhibition factor, NPBI; non-protein bound iron, RT-PCR; reverse transcriptase polymerase chain reaction, TH; total hydroperoxide.

[30], combining corticosteroids with surfactant as a vehicle has the potential to confer the desired benefits of corticosteroid therapy in the lungs, without the undesirable systemic side effects. The combination of corticosteroids with surfactant has been extensively investigated in basic science, pre-clinical animal models and, to a lesser extent, in human trials. Emerging evidence from randomized trials suggest a significant reduction in short- and long-term respiratory morbidity attributed to the addition of corticosteroids to surfactant [31,32].

3.5. Corticosteroids do not impair innate surfactant function

Although natural cholesterols are present in pulmonary surfactants [33], high levels are known to completely inactivate surfactant surface activity [34]. All steroid hormones are derived from cholesterol, and therefore it has been postulated that the cholesterol component of corticosteroids may interfere with innate surfactant function. Therefore, the impact of various concentrations of corticosteroids on innate surfactant function has been extensively investigated. The ratio of phospholipid to corticosteroid concentration, and the cholesterol content of the surfactant vehicle have an important influence on the impact of corticosteroids on innate surfactant function [20].

Nimmo et al. (2002) demonstrated that a concentration ratio of 250:1 bovine surfactant (Survanta, Abbott Laboratories, Abbott Park, IL) to dexamethasone did not alter the surface tension properties [35]. Use of up to 100:1 calfactant (Infasurf, ONY Incorporated, Amherst, NY) to budesonide and up to 10:1 calfactant to beclomethasone does not interfere with innate function [19,20]. Similarly, when mixed at a ratio of 10:1, beclomethasone and fluticasone also do not alter Prosurf (bovine surfactant, Nialtec S.A., Buenos Aires, Argentina) function [36]. However, increasing beyond these concentrations leads to early collapse of surfactant films [19]. In contrast, Curosurf (Poractant alfa, Chiesi Farmaceutici, Italy) is able to accommodate higher concentrations of corticosteroid, presumably due to its cholesterol-free preparation. Survanta is able to accommodate $\geq$50:1 surfactant:budesonide, and Curosurf $\geq$160:1 [37]. Concentrations beyond this will interfere with innate surfactant function [37,38]. As the impact of corticosteroids on innate surfactant is variable, it is important that innate surfactant function is thoroughly tested before implementation in human trials. These studies are detailed in Table 3.

3.6. Corticosteroids administered using surfactant vehicles are well distributed through the respiratory system

The earliest study examining the distribution of corticosteroids delivered using a surfactant vehicle was reported in 1998. In their study, Fajardo and colleagues demonstrated the effective distribution of radiolabelled budesonide after intratracheal administration using surfactant or normal saline as a vehicle [39]. Similarly, using radiolabeling techniques, Nimmo et al. (2002) demonstrated that when administered using a surfactant vehicle, dexamethasone was better distributed within the lungs compared to administration with a saline vehicle [35]. Recently, rodent studies have demonstrated that distribution of fluorescent dye after intratracheal administration was most extensive after combining it with budesonide and surfactant [40], and extensive distribution of radio-labelled budesonide when combined with either bovine (Survanta) or porcine (Curosurf) surfactants (Fig. 2) [37]. Details of these studies are shown in Table 4.

3.7. Corticosteroids have prolonged action and reduced systemic absorption when administered with surfactant

Direct pulmonary administration of corticosteroids carries the advantage of prolonged local action and reduced systemic absorption. Despite a relatively short plasma half-life, when administered into the lungs of adults and children, budesonide undergoes extensive intracellular esterification which results in prolonged release [41]. Nimmo et al. (2002) demonstrated that intratracheal administration of dexamethasone with a surfactant vehicle results in a 10-fold reduction in peak plasma drug concentration compared to IV administration in adult rats [35]. Whether intratracheal corticosteroids reach the brain remains in question. In a pharmacokinetic study of preterm lambs, budesonide metabolites were not detected in brain tissue 24 h after administration [42]. However, using quantitative RT-PCR analysis, Hillman et al. demonstrated intratracheal budesonide/surfactant administered at 0.25 mg/kg resulted in multiple altered gene pathways in the liver and periventricular white matter in preterm lambs [43]. Although doses lower than this may result in less systemic absorption, they do not confer the same pulmonary benefits [44]. Similarly, higher doses do not confer additional pulmonary benefit and have the increased risk of central nervous system absorption [45]. Although in-vivo metabolism of corticosteroids in preterm lambs and humans are not directly comparable [42], these findings highlight an important need for long term neurodevelopmental follow up of infants enrolled in clinical trials exploring these new therapies. Details of these studies are shown in Table 4.

3.8. Corticosteroids reduce lung injury in animal models of the preterm lung when administered with surfactant

Animal studies provide a valuable model for neonatal pathology, permitting the development of important translational evidence prior to implementation of therapies in clinical trials. The therapeutic benefits of intrapulmonary corticosteroids delivered via a surfactant vehicle have been demonstrated in a number of small and large animal studies. Beclomethasone combined with surfactant reduced markers of oxidative stress and inflammation in preterm lambs when it was administered before the onset of ventilation [46,47]. In saline induced RDS piglet models, budesonide and surfactant improved oxygenation, reduces proinflammatory cytokine expression and reduces histological lung injury, in particular after a longer duration of ventilation [48,49]. Similarly, budesonide administered via surfactant lavage reduces oxidative markers, neutrophil counts on bronchiolar lavage and cytokine mRNA expression compared to surfactant lavage alone in rabbit models of meconium aspiration syndrome [50]. Recently, it has been demonstrated that budesonide and surfactant increases lung maturity [45] and reduces lung injury and associated systemic responses in the liver and brain [51]. This effect is consistent when budesonide and surfactant are used after normal and injurious ventilation strategies [43,51]. Similar benefits in lung mechanics and lung inflammation are observed with budesonide and surfactant when administered to preterm lambs following intra-amnionitis exposure to lipopolysaccharide, replicating inflammation observed during chorioamnionitis, even when combined with injurious ventilation strategies [52]. Overall, data from animal studies suggests consistent pulmonary benefit for intratracheal corticosteroids and surfactant, providing encouraging evidence to support ongoing human trials. Details of these studies are shown in Table 5.

3.9. Human trials suggest improvement in pulmonary outcomes following intratracheal administration corticosteroids combined with surfactant

Building upon years of translational research, recent human trials have demonstrated promising evidence of improved short and long term important clinical outcomes by pulmonary corticosteroids delivered by surfactant vehicles in preterm infants. In 2008, Yeh et al. conducted the first randomized controlled trial assessing the effect of intratracheal budesonide and surfactant on a composite outcome of death or bronchopulmonary dysplasia (BPD). 116 very preterm infants born with a birth weigh of $<$1500g who were mechanically ventilated in $>$0.6 FiO_2 were randomized to receive either surfactant and budesonide or surfactant alone. Infants received repeated doses every 8 h until the FiO_2 was $<$0.40 or until extubation. In addition plasma cortisol, budesonide and 16α-hydroxyprednisolone levels were measured at 8 h post treatment. Treatment with budesonide and surfactant resulted in a

Table 6
Randomized controlled trials of corticosteroid delivered by a surfactant vehicle.

Author (year)	Population	Corticosteroid	Surfactant	Outcome	Results	Comments
Yeh (2008)	n = 116 **Intervention:** GA: 26.4 (2.2) BW: 881 (245) **Control:** GA: 26.7 BW: 919 (272)	Budesonide (2.5 mg/kg)	Survanta (100 mg/kg)	Composite outcome of death or BPD at 36 weeks PCA	Significant reduction in BPD in the intervention group (31.7 % vs 60.7 %, p = 0.003)	Also demonstrated a 10-fold reduction in systemic absorption compared to IV administration Also measured in-vitro impact of budesonide and surfactant
Yeh (2016)	n = 265 **Intervention:** GA: 26.5 (2.2) BW: 882 (249) **Control:** GA: 26.8 (2.2) BW: 935 (283)	Budesonide (2.5 mg/kg)	Survanta (100 mg/kg)	Composite outcome of death or BPD at 36 weeks PCA	Significant reduction in BPD in intervention group (42 % vs 66 %, RR 0.58, 95 % CI 0.44–0.77, p < 0.0001). Intervention arm had significantly lower interleukin levels in tracheal aspirates at 12 h, 3–5 and 7–8 days.	Multiple doses of budesonide and surfactant given (up to 6) in a population at high risk of BPD

BPD; bronchopulmonary dysplasia, BW; birth weight, CI; confidence interval, GA; gestational age, PCA; post conceptual age, RR; risk ratio.
All data mean (standard deviation) unless otherwise specified.

significant reduction in a composite outcome of death or BPD (31.7 % vs 60.7 %, p = 0.003). Survival free of BPD was greater in infants treated with budesonide and surfactant (82 % vs 58 %, p = 0.025). Respiratory support requirements including mean airway pressure (MAP), fraction of inspired oxygen (FiO_2), and oxygenation index were lower in the intervention group. Plasma concentrations of budesonide and 16α-hydroxyprednisolone peaked at 30 min and 2 h respectively. Approximately four percent of budesonide was absorbed into the systemic circulation. The same investigators have subsequently performed a larger randomized control trial in 265 very low birth weight infants (VLBWI, birth weight <1500 g) at high risk of BPD. Infants were included if they had severe RDS, required mechanical ventilation and/or had an FiO_2 of ≥0.6. Infants received repeated doses of either surfactant and budesonide or surfactant alone 8 hourly until the FiO_2 was <0.3 (maximum six doses) or they were extubated. Treatment with budesonide and surfactant again resulted in a signification reduction in the primary composite outcome of BPD or death (42 % vs 66 %, RR: 0.58 [95 % CI: 0.44–0.77]) with a corresponding number needed to treat of 4.1. Tracheal aspirates were collected and interleukin (IL) levels measured in 18 and 20 infants in the intervention and control arms, respectively. Significant reduction in IL-1, IL-6 and IL8 were observed in the intervention group. Ongoing assessment of long term follow up is underway. Details of these studies are shown in Table 6. At present, three large randomized controlled trials are ongoing (Preventing Lung Disease Using Surfactant + Steroid (The PLUSS Trial); anzctr.org.au Identifer: ACTRN12617000322336, The Budesonide in Babies (BiB) Trial (BiB); ClinicalTrials.gov Identifier: NCT04545866 and Routine Administration of Surfactant/Budesonide to Prevent BPD in VLBW With RDS: A Double Blind Study; ClinicalTrials.gov Identifier: NCT03275415), which in combination are recruiting over 2500 infants and will further add robust clinical evidence for this emerging therapy.

4. Conclusion

In conclusion, this chapter has explored the use of surfactant vehicles as a promising drug delivery system for targeted therapeutic administration. We have detailed the unique molecular characteristics of surfactant and its physical properties that make it a powerful vehicle for drug delivery. Desirable characteristics of drugs incorporated into surfactant, including preserving innate surfactant function while conferring additional therapeutic benefits have also been explored. A wealth of preclinical animal data underpins the incorporation of corticosteroids into surfactants to mitigate lung injury. As this research field continues to gain traction, the future holds promise for the wider application of innovative combinations of surfactant vehicles and additional therapeutic agents, which may warrant further exploration in human trials. Finally we have reviewed randomized controlled trials of intrapulmonary corticosteroids delivered using surfactant vehicles. Current evidence suggests a benefit for intratracheal corticosteroids delivered by surfactant vehicles in improving meaningful clinical outcomes with several large trials currently recruiting and nearing completion. By continuing to build upon the existing body of evidence, and following a robust research pipeline of benchtop studies, animal research, and subsequent human trials, this groundbreaking drug delivery approach holds the potential to revolutionise neonatal medicine in the years to come.

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