Steroids, surfactant and lung disease

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The paper by Wang and co-workers¹ in this issue of *Thorax* (pp 907-13) demonstrates a direct effect of postnatally administered dexamethasone on airway concentrations of surfactant proteins in preterm infants with idiopathic respiratory distress syndrome (IRDS). Compared with a control group, concentrations of the surfactant specific proteins SP-A and SP-D increased in tracheal aspirate samples during a 14 day period of dexamethasone treatment, while the albumin concentration concomitantly decreased. The authors propose that increased expression of SP-A and/or SP-D may be one mechanism of action when dexamethasone is used in attempts to prevent or palliate neonatal chronic lung disease. This paper is important and raises a number of intriguing questions, both about the role of surfactant components in postnatal lung disease and about the mode of action of glucocorticoid hormones in the lungs.

There are limitations to this study which the authors acknowledge. Expression of results as absolute concentrations does not allow for variable aspirate dilution, but calculation relative to the variable concentration of albumin was evidently not appropriate. The authors only measured the hydrophilic protein components of surfactant, and provide no information about the phospholipid components which contribute predominantly to the surface properties of surfactant. Consequently it is not clear whether the steroid effect shown here was specific for surfactant proteins, or whether it represented a more general elevation of all surfactant components. Overall stimulation of surfactant production cannot be assumed, as increasing evidence suggests that the secretion of surfactant phosphatidylcholine and SP-A are regulated independently.² Nevertheless, this remains the first description of an effect of early administration of postnatal glucocorticoids on surfactant protein components. Moreover, given the widespread adoption of exogenous surfactant therapy for treatment of IRDS in recent years, this study is especially valuable as it could not now be repeated.

At first sight it is not surprising that dexamethasone may alter surfactant production in newborn infants, given the proven benefits of prenatal administration of glucocorticoid hormones on fetal lung maturation. However, dexamethasone acts on many processes in the developing lung, and the precise details of its actions are dependent both on concentration and stage of development. For instance, prenatal administration of dexamethasone to pregnant rats decreases the rate of fetal lung growth and accelerates lung differentiation, shown by increased numbers of type II alveolar epithelial cells and increased synthesis and secretion of surfactant phospholipid.³ In contrast to this prenatal effect, postnatal dexamethasone administration at 14 days of age to preterm infants at risk of chronic lung disease did not augment surfactant phospholipid composition, assessed by the fractional concentrations of phosphatidylglycerol or dipalmitoyl phosphatidylcholine in lung lavage samples.^{4,5} Variable expression of SP-A by organ cultures of human fetal lung has been reported in response to dexamethasone, attributed to increased transcription and decreased stability of the mRNA for this protein.⁶ Synthesis of SP-A in rat lung was only stimulated by dexamethasone in the early postnatal period, and not at

older ages. Similarly, in human preterm infants given dexamethasone from 14 days of age, SP-A concentration in lung lavage samples only increased transiently for the initial day of treatment and then returned to control values.¹

All these considerations suggest that any effect of glucocorticoid hormones on expression of surfactant proteins is likely to be greatest in the immediate postnatal period, and that such a mechanism probably does not contribute to the therapeutic response to steroids in the mature lung. Furthermore, the significance of the contribution of increased synthesis of SP-A and SP-D to the improved lung function in response to dexamethasone in preterm infants is by no means clear, given the wide range of additional modes of action of steroids. These include decreased recruitment of inflammatory cells into the lungs, inhibition of secretion of inflammatory mediators such as prostaglandins and leukotrienes, and decreased elastase concentration. While dexamethasone is established as an effective treatment for established chronic lung disease, its role in the immediate postnatal period is more contentious. For instance, one recent study showed no clinical benefit when dexamethasone was given prophylactically over the first three days of life to preterm infants, and longer treatment schedules carry an increased risk of adverse effects.⁷ Such effects include enhanced risk of infection, decreased growth, and reduced alveolus formation. These complications are of less immediate importance in established chronic lung disease where there are few alternative treatments for a severe life threatening condition. They become more significant with early use of dexamethasone to prevent the inflammatory insult and reduce the incidence of chronic lung disease, as only a minority of preterm infants with IRDS will develop chronic lung disease.

With respect to surfactant function in the perinatal period, the roles for SP-A and SP-D are also unclear. In normal fetal lung development the synthesis, storage, and secretion of surfactant phospholipid increases considerably towards term. Moreover, the severity of RDS in preterm infants correlates with the magnitude of the deficiency of SP-A rather than that of disaturated phosphatidylcholine.⁸ In contrast, all currently available exogenous surfactant preparations for the effective treatment of RDS are based either on lipid extracts of natural animal surfactants or are completely synthetic. Neither type of surfactant contains either SP-A or SP-D. In summary, RDS in preterm infants is associated with deficiency of SP-A as well as of surfactant phospholipid, and the improvement of lung function in response to dexamethasone is associated with increased tracheal aspirate concentrations of SP-A and SP-D rather than of phospholipid. However, the considerable clinical benefits of exogenous surfactant therapy to preterm infants with RDS are due to preparations which contain neither SP-A or SP-D.

This dichotomy between information about specific components of surfactant and understanding of the overall contribution of surfactant to the maintenance of optimal lung function is also apparent in a wider context. One problem here is the large number of proposed roles for surfactant within the lungs. While prevention of atelectasis and maintenance of alveolar patency are well established, additional roles include prevention of airway oedema,

plugging and collapse, promotion of ciliary action, an anti-oxidant capacity, and multiple host defence effects.⁹ Moreover, surfactant is a functional description of a heterogeneous material defined as the fraction of a lung lavage sample that, under surface area compression in an appropriate surface tension balance, can reduce surface tension to near zero. It is an insoluble phospholipid:protein complex which is secreted as large, surface active aggregates and undergoes a complex intra-alveolar metabolism into small, unilamellar vesicles which are inactive and are largely recycled by uptake into type II alveolar epithelial cells. Some components, such as the phospholipids and hydrophobic proteins SP-B and SP-C, are integral and essential for surface activity, while others such as SP-D apparently are not.

The multiple functions suggested for surfactant have largely been investigated using purified components. For instance, surfactant phospholipid is apparently largely anti-inflammatory and inhibits both lymphocyte proliferation and the respiratory burst of neutrophils. In contrast, SP-A and SP-D exhibit considerable pro-inflammatory effects, binding and opsonising Gram negative bacteria, promoting alveolar macrophage functions, and binding to pollen granules and other aeroallergens. It is tempting to speculate that the balance between the phospholipid and hydrophilic protein components of surfactant may contribute to the magnitude of the inflammatory response to, for instance, inhaled pathogens, though little direct experimental evidence exists to support or refute this concept. Functional interactions between surfactant components have been extensively studied in respect of surface tension function but have not yet been investigated with regard to host defence responses. For instance, purified SP-A binds to numerous pathogens and promotes alveolar macrophage phagocytosis, but there is negligible free SP-A in the lungs and it is not clear yet whether phospholipid-bound SP-A can exert a comparable spectrum of actions.

Extensive information is available about the molecular biology of surfactant proteins, the regulation of synthesis of surfactant phospholipid, and the cell biology of surfactant secretion and turnover. In contrast, there is little definite information about the contribution of surfactant dysfunction in diseases of the mature lung. Part of this is historical, surfactant being viewed by respiratory physicians as the province of the neonatologist because of the clearly defined role of surfactant deficiency in the aetiology of IRDS. Subsequently, deficiency of SP-B has been identified as one primary cause of congenital alveolar proteinosis in term infants,¹⁰ and abnormal surfactant function is clearly apparent in adult alveolar proteinosis. Inhibition of surface activity of surfactant has been proposed as a major contributor to the pathogenesis of adult respiratory distress syndrome, but clinical trials in this condition with the synthetic surfactant Exosurf have not been encouraging.¹¹ Proposed contributions of alterations in specific surfactant functions to the lung problems in asthma, cystic fibrosis, congenital diaphragmatic hernia, air pollution exposure, and bacterial pneumonia have not yet been adequately elaborated.

One limiting factor here is the lack of standardisation and availability of appropriate techniques with adequate sensitivities to quantify surfactant components in limited volumes of bronchoalveolar lavage samples available from clinical studies of disease states. Availability of antibodies has facilitated quantitation of SP-A and SP-D, as in the study by Wang *et al.*,¹ but the wide variation in values between different groups probably reflects problems associated with antibody binding to heterogeneous lipid:protein complexes in comparison with purified protein standards. Measurement of SP-B and SP-C is more difficult as

these lipid-soluble proteins are poorly immunogenic and established ELISA techniques are very insensitive. Most studies of phospholipid composition still rely on insensitive thin layer chromatography techniques, supplemented with measurement of disaturated phosphatidylcholine as the residue after destructive oxidation of unsaturated phosphatidylcholine species using osmium tetroxide. Several more specific and sensitive techniques have been developed, based on high performance liquid chromatography, mass spectrometry or NMR, but have yet to achieve widespread use in clinical studies. Some degree of standardisation may result from the task force set up by the European Respiratory Society to report on the current status of techniques for assay of acellular components in bronchoalveolar lavage samples, which is due to report towards the end of the year.

Hopefully the standardisation of analyses of lung surfactant composition and functions will facilitate the recognition of the contribution of this complex material to the maintenance of normal lung function, as well as to disease. It is very unlikely that, for instance, disruption of surfactant function will be identified as a major initiating factor in either asthma or cystic fibrosis, but it may well contribute to the severity of the subsequent disease process. Air pollutants, both gaseous and particulate, interact initially with the surfactant layer at the air:liquid interface in the lungs, and integrity of the surfactant system has been proposed as a major protective factor against oxidant-induced lung damage. All cellular reactions in the alveolus and airways take place within a surfactant enriched environment, and interaction of surfactant with cells must be considered in interpreting their various responses. At the very least, the adequacy of surfactant composition and function should be considered as one potential contributing factor in studies of diseases of the mature as well as the neonatal lung. One feature of such studies must be an integrated approach to determine the wide spectrum of surfactant components and functions in order to establish the extent of the contribution of surfactant dysfunction to respiratory diseases.

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