Pulmonary surfactant: unanswered questions

The story of pulmonary surfactant is, in many ways, another of the many recent triumphs of medical science – a field that was first fully recognised three decades ago has matured to the point where it now has a major impact on clinical practice, saving thousands of neonates each year. The existence of surfactant, predicted by von Neergaard in 1929, was demonstrated in the late 1950s by Pattle[1] and Clements,[2] marking the beginning of the era of surfactant research. There followed shortly afterwards the demonstration by Avery and Mead that the lungs of premature infants with neonatal respiratory distress syndrome were deficient in surface tension lowering ability, soon to be recognised as deficit of surfactant that was due to immaturity of the surfactant secreting apparatus.[3] When the peculiar lipid composition of lung surfactant was established[4 5] it became possible to predict neonatal respiratory distress before birth by a low lecithin/sphyngomyelin ratio in amniotic fluid,[6] marking the application of basic science to clinical practice in this field. The discovery that corticosteroids could accelerate the maturation of premature lungs in terms of surfactant secretion[7] was the first therapeutic bounty of this new understanding. Over this and the next decade – a period of consolidation – most of the basic aspects of surfactant biology were put in place. The type II cell was recognised as the source of surfactant and isolated and cultured in vitro,[8] an art that is still not perfect but which has revealed much about surfactant synthesis, release, and metabolism. The biophysical properties of surfacant were explored,[9] its apoproteins were discovered, and its ultrastructural forms were revealed.[10] Every aspect of this work has been astonishing, from the fact that type II cells can secrete almost their weight in surfactant every day, to the fact that surfactant reduces the surface tension of the alveolar aqueous lining layer to zero (or very close), to the fact that the low molecular weight surfactant apoproteins are the most hydrophobic proteins known, to the amazing ultrastructural beauty of tubular myelin and the almost incomprehensible way such structures could be both derived from the bland concentric lipid layers of lamellar bodies and evolve into a phospholipid monolayer at the air-fluid interface, as is now believed.[11] Like most exciting areas of biological research, it helps to have an understanding of biochemistry, physiology, cell biology, biophysics, and now, molecular biology, to appreciate fully its development.

In the last 5–10 years surfacant replacement therapy for premature neonates has captured attention because of its dramatic effect on survival. To illustrate this, two forms of surfactant for replacement therapy – one natural and one synthetic – were approved for general use in the USA in 1990. In the next two years there was an overall decrease in neonatal mortality of 15% – overall, not just in the premature neonatal population. If one considers only the group at high risk for neonatal respiratory distress syndrome – that is, those infants of less than 29 weeks or 1200 g – the effect has been much greater, perhaps 50%.[12]

One could be excused for concluding that the progress from discovery of surfactant deficiency as a major cause of mortality in premature infants to its successful treatment in a few decades signals the end of the story. But it is certainly not the end of our understanding of surfactant biology and it could be just the beginning of its full clinical potential.

To deal with its clinical application first, two problems stand out. The optimal form of surfactant to use for replacement in premature neonates has yet to be identified and made available in an inexpensive form for widespread use. The natural surfactants used for replacement therapy come from bovine or porcine lungs. In addition to the phospholipids, they contain both low molecular weight surfactant-associated proteins – SPB and SPC – which have important roles in making the surfactant rapidly spreadable and in stabilising the film at the air-fluid interface. However, natural surfactant is not very abundant, even in cows, and requires extensive processing and sterilisation. For these reasons it is expensive – in the USA the one natural surfactant that is available costs about $500 per treatment for the surfactant alone and a premature infant will usually receive at least two doses. It is much simpler and cheaper to make a synthetic mixture of phospholipid(s) with or without spreading agents. Synthetic surfactants, of course, lack the protein components that are felt to be important for normal surfactant biophysics, and they do not behave exactly as natural surfactants in vitro. Making the SPB and SPC by genetic engineering so that they could be added to a phospholipid cocktail is complicated by the fact that these proteins are so hydrophobic. The technology could probably be developed but the expense would be such that the product would be likely to cost more than a natural surfactant. It might, however, be possible to devise synthetic substitutes for SPB and SPC, perhaps peptides that could be synthesised in vitro, and there is an ongoing search for such possible peptides.

In practice there does not seem to be much difference in efficacy between natural and synthetic surfactants, as reviewed by Jobe.[12] The incidence of respiratory distress syndrome complications and mortality tends to be less with natural surfactants, and perhaps this could be improved upon at a lower cost with better synthetic surfactants.

Other details of surfactant replacement in premature neonates still have to be worked out. Should one use replacement therapy routinely in premature infants or only when respiratory distress syndrome occurs? If prophylactically, how many doses should be given? These questions will probably be answered by experience in the next few years.

The next and more vexed question concerns adult respiratory distress syndrome (ARDS), a much more complicated problem that was reviewed in *Thorax* in 1990.[13] Because of its striking efficacy in neonatal respiratory distress syndrome surfactant administration has been considered for adult respiratory distress syndrome too. Obviously the pathophysiology of the two conditions is quite different; indeed, there could be as many patho-

physiological mechanisms of ARDS as there are causes. In contrast to neonatal respiratory distress syndrome, ARDS is characterised more by inactivation of surfactant than by insufficient production.[14] In ARDS the major problem seems to be that excessive microvascular leakage of circulatory fluid and proteins results in alveolar oedema which prevents gas exchange in some lung units that are still perfused. Although surfactant production is usually only moderately reduced in most experimental forms of ARDS, hyperoxia being a more extreme case,[15] a more frequent and serious physiological problem seems to be that surfactant function is severely compromised by the leakage of circulatory proteins into the alveolar air spaces. Many of these proteins, in particular fibrinogen and its products, react with surfactant and destroy its surface tension lowering properties.[16] The mechanical problem in ARDS – low lung compliance – thus has more to do with inactivation of surfactant than with deficient surfactant production (although the latter probably occurs and has been demonstrated in human ARDS[17]). Surfactant administration in ARDS would thus have, at the very least, to overcome all the inhibitory effects of ectopic alveolar exudate before any amelioration in surface forces would occur. Despite some small clinical trials with promising outcomes – for example, a trial of the natural surfactant Survanta in the USA[18] – a critical trial in ARDS that includes sufficient patients to yield a confident result is lacking at present. Nor, if one were to be successful, is it easy to envisage that surfactant replacement in its present form could be widely implemented for ARDS. Consider that the adult lung is about 100 times the size of a premature neonatal lung, and that more surfactant would be required to overcome the inhibitory effects of massive capillary leakage than to replace normal surfactant production, then one can appreciate that very large amounts of surfactant – perhaps three orders of magnitude more than in neonatal respiratory distress syndrome – would be required to correct the low compliance of the surface element in ARDS. Moreover, instead of the usual 2–4 treatments in neonatal respiratory distress syndrome, treatment in adults would probably have to be prolonged. This presents an insupportable economic cost, hence an even greater need for an inexpensive synthetic surfactant. Perhaps a more effective means of administering exogenous surfactant – for example, by aerosolisation – could reduce the amount needed to treat adult lungs. However, a large controlled trial of the synthetic surfactant Exosurf yielded no benefit in physiological measurements during the five days of treatment, nor any difference whatsoever in 30 day mortality.[19] Serious problems therefore remain with both cost and efficacy in the application of surfactant replacement therapy in ARDS.

Are there other clinical conditions in which surfactant abnormalities are present, and which might be amenable to "surfactant therapy" of an unspecified form? The answer is almost certain to be affirmative. If one considers the question from a physiological standpoint, small airways need a fluid lining layer of low surface tension for the same reasons as do alveoli. It may be that the pathophysiology of some airways diseases includes a component due to some unrecognised surfactant abnormality. Investigations along these lines have been pursued in asthma, but these have not been fruitful so far. Considering the question from a basic standpoint, there is a maxim that for every protein in the human there probably exists at least one disease due to genetic abnormality of that protein. Surfactant contains at least three proteins as integral components of its structure; diseases corresponding to mutations of each of these must occur. What are they? SPA exists in more than one allelic form but the known alleles appear to be neutral.[20 21] Deleterious genetic aberrations of SPA are not known at present; some may be lethal in neonates but there may well be others that contribute to clinical pulmonary disease and that await discovery. A mutation of the SPB gene that causes a lethal neonatal form of alveolar proteinosis has recently been recognised and described.[22] A fuller evaluation of its abnormal function may well provide us with clues to the mechanisms of the adult form of this perplexing condition in the same way that mutations of the α_1-antitrypsin gene have not only explained a rare disease but have also provided us with a paradigm for the pathophysiological mechanism of "garden variety" emphysema. The tools for genetic analysis of this sort are more or less routine and cry out for wider exploitation in respiratory disorders – a painstaking process but one that is bound to yield fascinating and fruitful insights into clinical pulmonary medicine.

Let us now consider some current questions about surfactant biology. Many details of the intracellular metabolism of surfactant components have been worked out and reviewed in recent years.[11] Further studies in this area would be greatly facilitated if "normal" type II cells could be maintained in a fully functional form in vitro, but these cells are recalcitrant and refuse to conform phenotypically when they are separated from their usual environment. This problem has not been solved. We still do not know all the details of the control of surfactant secretion and reuptake in type II cells. Another major area of ignorance is the structure of the various forms of surfactant after it is secreted. We understand that surfactant is secreted as lamellar bodies and evolves sequentially through a tubular myelin form, a surface form (the monolayer), and a small vesicular (micellar) form, and is then largely taken back into type II cells for recycling. However, the details of this sequence and alternative routes for surfactant clearance from the alveoli are not well understood. We have recently shown that a serine protease that is presumed to be secreted with surfactant by type II cells is required for the conversion of tubular myelin to small vesicles.[23 24] Although this enzyme does not seem to be important to the physiological function of surfactant,[25] its function, substrate(s), and cellular source have still to be determined.[26] Being a serine protease, its action is inhibited by the serine protease inhibitor α_1-antitrypsin at concentrations of the latter that are normal in the alveolar fluid lining layer. This raises the possibility that α_1-antitrypsin plays a part in surfactant metabolism in addition to its well known role in lung defence.[27] The role of an enzyme in the extracellular metabolism of surfactant is another surprise in the surfactant story, and its importance in human health and disease is entirely unknown.

Equally enigmatic at present is the molecular structure of surfactant itself. Electron micrographs of its various structural forms reveal phospholipid membranes, presumed to be ranks of phospholipid molecules arrayed shoulder to shoulder as in the unit membranes of cells, but we have only the sketchiest notion of where the various surfactant apoproteins fit into these structures, or how they affect the surface tension properties of surfactant structures, or how the phospholipids and apoproteins are arranged and rearranged as surfactant evolves from one structural form into another. Innovative molecular techniques are being brought to bear on these questions and it will probably not be very long before a more complete picture emerges. Again, this will undoubtedly be fascinating and may bear on clinical pulmonary disease.

The surfactant story, although demonstrating major achievements in advancing clinical practice, clearly promises to yield many more surprises and dividends as the

obvious questions are answered and new questions are raised.

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