Serotonin: a new start for an old friend?

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Understanding the mechanisms of fibrotic lung disorders, either idiopathic or associated with a specific aetiology, is the subject a huge scientific effort in the world, sustained by both the academic pulmonary community and by pharmaceutical companies. The ultimate aim is clearly to identify one or more drugs that will have the capacity to inhibit the decline of lung function and improve survival. Such ambitious goals are unlikely to be reached from one day to another. Rather, we may expect small improvements, perhaps in subgroups of patients, which will add to improve globally the prognosis of this disease process. In the modern era of medicine, in a time in which the transmission of information is rapid and global, such a slow pace of evolution is difficult to accept and certainly contributes to the sense of nihilism sometimes affecting the respiratory community at large, including clinicians, patients and their families, who are challenging fibrotic lung disorders.

This negative feeling is also fuelled by the litany of clinical trials in patients with idiopathic pulmonary fibrosis, with negative results (trials evaluating the effect of etanercept,[1] imatinib[2] or bosentan,[3] for example), with conflicting results such as the recent CAPACITY pirfenidone trials,[4] or with minimally positive results as with sildenafil.[5] Why so many negative results? Before reaching patients, most of these molecules have been tested positive in animal models of lung fibrosis, particularly in the bleomycin-induced lung fibrosis model in rodents. This model is widely used in the scientific community to identify therapeutic targets for treating human idiopathic pulmonary fibrosis, and the question remains whether the model is the right model, or whether we should move to a better model for human disease.[6] Because of the questionable value of that model, it is absolutely necessary to confirm all results obtained in animals by data demonstrated in situ in human lung samples. This is an important point to understand the potential value of the results presented by Konigshoff and colleagues[7] in this issue of the journal. These authors should be congratulated for bringing together compelling data supporting the antifibrotic action of terguride, a 5-hydroxytryptamine 2A (5-HTR_{2A}) and 5-hydroxytryptamine 2B (5-HTR_{2B}) receptor antagonist. They demonstrated that these receptors are overexpressed in the fibrotic lung in humans, and that this inhibitor limits the development of fibrosis in bleomycin-induced lung fibrosis in mice in vivo and limits the collagen production induced by transforming growth factor beta(1) on human lung fibroblasts in vitro. These results support previous studies published 2 years ago by our group, showing that the pharmacological blockade of either 5-HTR_{2A} or 5-HTR_{2B} reduced bleomycin-induced lung fibrosis in mice and promoted an antifibrotic environment by decreasing the expression of profibrotic mediators, namely transforming growth factor beta (1), connective tissue growth factor and plasminogen activator inhibitor 1 messenger RNA, but had minimal effects on lung inflammation as assessed by bronchoalveolar lavage cytology analysis.[8] Interestingly, these receptors are widely distributed in the fibrotic lung, as endothelial cells, epithelial cells and fibroblasts (in particular, we observed that fibroblastic foci specifically expressed the 5-HTR_{2B} receptor)[8] are positively stained with available antibodies, suggesting that all these cell types could be targets for the drug. As a direct effect of terguride on fibroblasts is demonstrated in the paper by Konigshoff and colleagues,[7] it would be very interesting to determine whether $5\text{-HTR}_{2A/2B}$ inhibition could also target alveolar epithelial cells, perhaps by modulating the process of epithelial—mesenchymal transition, which is believed to play a role in lung fibrosis.[9]

Serotonin (also known as 5-hydroxytryptamine) is a peptide with well-known properties in the lung.[10] Serotonin is synthesised from tryptophan and pooled in platelets, which store and release serotonin by its serotonin transporter (5-HTT). Serotonin exerts its action through seven different receptors (5-HTR_1 to 5-HTR_7). With the exception of the 5-HT_3 receptor, a ligand activated ion channel, all other serotonin receptors are G protein-coupled receptors. Very low levels of circulating free serotonin are detected in normal conditions as serotonin is rapidly degraded by the monoamine oxydase A. In the fibrotic lung, different sources of serotonin may contribute to the local burden of this mediator, platelets obviously, but also neuroendocrine cells[11] and mast cells,[12 13] which are both increased in the fibrotic lung, and endothelial cells, which have recently been shown to secrete serotonin.[14]

The demonstration that serotonin is implicated in lung remodelling is not new as serotonin has previously been shown to regulate the remodelling of pulmonary arteries in various forms of pulmonary hypertension,[15] focussing either on the role of serotonin receptors[16] or serotonin transporters.[17] Interestingly, its profibrotic role has also been suggested in the tissue remodelling associated with cancer,[18] or in other fibrotic disorders, such as liver fibrosis.[19]

Altogether, these results suggest that targeting serotonin could bring fresh air to the treatment of fibrotic lung disorders. Is it time for clinical trials?

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Exosomes in lungs of patients with sarcoidosis: a contributor to immune pathogenesis or just another by-product of heightened immune activity?

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The concept that cells can directly communicate with, and influence the function of other cells by transfer of particulate complexes or cell surface proteins (eg, antigen-bound MHC-II, integrin, ATPase channels)[1–3] rather than soluble factors like cytokines and chemokines has excited cell biologists for decades. Extensive efforts have been made to prove the existence of this phenomenon and understand the mechanisms by which cells (especially immune cells) transfer proteins between each other. There is now evidence for at least four ways that this transfer could occur[4]—proteolytic cleavage of the protein from one cell with attachment to another, formation of tubules between two cells, direct cell membrane fusion and transfer of enclosed membrane vesicle (figure 1). An exosome is an example of such a membrane vesicle and is significant in that it can contain the contents of both intracellular endosomes and proteins expressed on the cell membrane of its parent cell. Therefore, it could be viewed as a 'mini-cell', but with the added capacity to transfer the cell content or surface proteins onto another cell.

With the acknowledgement that exosomes exist and can transfer cellular material, the focus has shifted to showing that this phenomenon has functional consequences. Interest was roused when several investigators began showing that peptide—MHC complexes on exosomes can be captured by dendritic cells, which then trigger CD4 and CD8 T-cell responses.[5–7] Depending on the kind of T cells engaged by these complexes, the result could be amplification of the T-cell response or suppression, for example, if regulatory T cells were involved. Therefore, exosomes could also influence the net outcome of a lymphocytic response during infection or inflammation. The ability of exosomes to trigger immune response has been utilised in the field of tumour immunology. At least two phase I clinical trials have been carried out using exosomes to enhance the body's own immune response against tumour cells.[8 9] Morse et al[9] purified autologous, dendritic cell-derived exosomes expressing MHC-II and used these as platforms for loading of tumour-specific antigen. They showed that infusion of these autologous exosomes was safe and resulted in detectable tumour-specific T-cell response.

However, it is widely acknowledged that the role of exosomes in vivo requires further clarification. Production of exosomes is widespread and the factors controlling its relative concentration in one inflammatory setting compared to another are poorly understood. In this edition of Thorax, Qazi and colleagues[10] (*see page 1016*) show that exosomes can be purified from the bronchoalveolar lavage fluid of sarcoidosis patients and that they are enriched compared to healthy controls. The study utilised multi-imaging modalities to show their presence and, more significantly, demonstrate that these exosomes were able to induce production of cytokines from peripheral mononuclear cells and epithelial cells. This finding forms the first step in explorations of exosomal function in lung disease. Many questions can now be raised—what is/are the parent source(s) of these exosomes? Does increased amount of exosomes contribute to amplification of the CD4 T-cell response observed in sarcoidosis? Is this a sarcoidosis-specific finding or are lungs of patient with asthma, COPD and idiopathic pulmonary fibrosis also enriched with exosomes? And do different diseases have exosomes that bear different cellular proteins? Do

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