Highlights from the symposium of the Society for Medicines Research, held March 14, 2001, in London, U.K.

Smoking-Related Lung Disease: Prospects for New Drug Therapy

by Trevor T. Hansel

Summary

The first 2001 meeting of the Society for Medicines Research, held in London on March 14, was devoted to emerging treatments for chronic obstructive pulmonary disease, commonly referred to as smoking-related lung disease or COPD. As COPD afflicts more and more people around the world, the dual need for preventative measures and therapeutic approaches will also continue to grow. During this sobering yet inspiring symposium, researchers looked at compounds that are currently in phase III clinical studies; reactive oxidant species, proteases and neutrophil chemotactics as therapeutic targets; and finally, the potential of monoclonal antibodies and retinoids.

The Smoking-Related Lung Disease (COPD): Prospects for New Drug Therapy symposium, organized by the Society for Medicines Research (SMR) was held March 14, 2001, at the National Heart and Lung Institute (NHLI) in Chelsea, London, U.K. The meeting involved an audience of some 80 participants in a one-day meeting that offered eight lectures on novel therapeutics for smoking-related chronic obstructive pulmonary disease (COPD). The three sessions of the meeting were chaired by Clive Page (Sackler Institute of Pulmonary Pharmacology, London, U.K.), David Cavalla (Arachnova, Cambridge, U.K.) and Trevor Hansel (National Heart and Lung Institute, London, U.K.). The meeting first considered compounds in phase III clinical studies for COPD—**tiotropium**, **AR-C68397AA** and **cilomilast**—before considering reactive oxidant species, proteases and neutrophil chemotactics as therapeutic targets. The final sessions looked to the exciting potential of monoclonal antibodies and retinoids to retard and even reverse the natural history of loss of lung function in COPD.

Overview of COPD

Peter Barnes (Dept. of Thoracic Medicine, National Heart and Lung Institute, London, U.K.) began the meeting by providing an overview of the pathological and immunological basis of COPD that has resulted in the escalating clinical and economic burden of this increasingly prevalent disease. Indeed, in 1990 COPD was the sixth most important cause of mortality in the Global Burden of Disease Study; it is projected to be third by 2020. It was noted that the public profile of asthma is very high, while COPD remains very much the poor relation to asthma, despite a glaring statistic of 26,033 deaths from COPD compared with 1,791 deaths from asthma in the United Kingdom in 1992. For the sake of medical and pharmaceutical advancement and public awareness, more research needs to be done on why only a minority of subjects

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develop COPD after prolonged cigarette smoking.

Prof. Barnes stressed that COPD and asthma are separate diseases that do not belong to a single spectrum of lung disease, as propounded in the Dutch hypothesis. COPD involves exposure to cigarettes; has an inflammatory response involving macrophages, neutrophils and CD8+ T cells; has a lack of airways hyperreactivity (AHR); and is a disease that does not respond to steroids. In contrast, asthma is frequently triggered by allergens, viral infections and exercise; has an inflammatory infiltrate of Th2 cells and eosinophils; has conspicuous AHR; and generally responds well to steroids. The anatomical location of COPD involves both destruction of lung parenchyma (emphysema) and inflammation of the small airways (chronic obstructive bronchiolitis). The architecture of the lung is destroyed by proteases (e.g., neutrophil elastase and matrix metalloprotease-9 [MMP-9]) and reactive oxygen species released from macrophages and neutrophils, as well as by the direct cytotoxic effects of activated CD8+ T cells.

In COPD there is loss of alveolar function in exerting tension on bronchiolar walls, in the manner of guy ropes upon a tent, contributing to the obstruction of small airways. Assessment of COPD is based firstly on spirometry, since the classic studies of Fletcher and Peto (1977) have elegantly delineated the accelerated loss in FEV_1 (forced expiratory volume in one second) that occurs in smokers who are susceptible to developing COPD. This is a fixed obstruction that is only partially reversed by inhaled bronchodilator therapy. A recent development is the demonstration of abnormalities—elevated levels of interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), growth-related oncogene-α (GRO-α) and leukotriene B_4 (LTB_4)—in induced sputum in COPD subjects compared with healthy smokers.

Prospects for novel therapies for COPD are based on consideration of the cells, mediators and enzymes involved in pathogenesis (Fig. 1). Cigarette smoking causes a major burden of oxidants, including superoxide anion, hydrogen peroxide, hydroxyl radicals and peroxynitrite. This burden causes an inflammatory response that is characterized by an amplification or exaggeration of response when comparing COPD patients with healthy smokers. Promising therapies include phosphodiesterase (PDE) antagonists, LTB_4 antagonists, chemokine receptor antagonists, TNF-α antagonists and elastase and matrix metalloprotease (MMP) inhibitors.

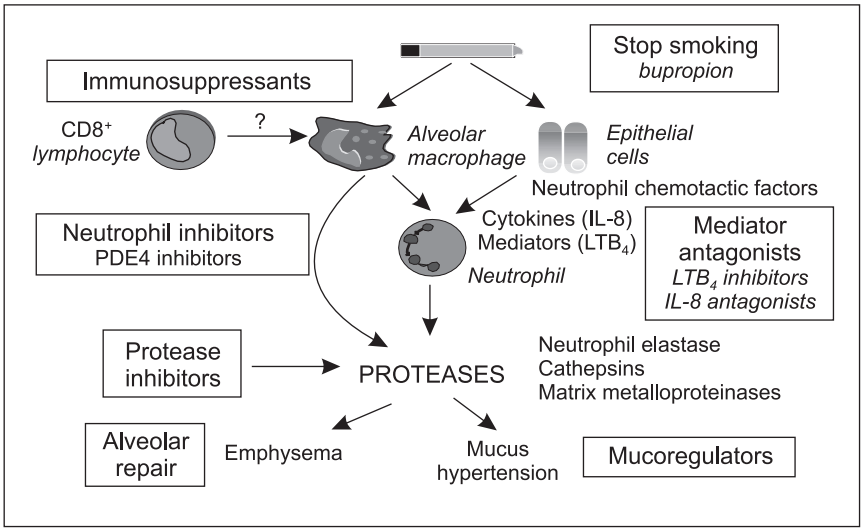

Fig. 1. Targets for COPD therapy.

An increasing array of methods are available for the diagnosis and monitoring of COPD. Potential clinical investigations include spirometry (FEV_1/FVC); assessment of reversibility to inhaled the β_2-agonist **salbutamol**; a trial of oral steroid to document lack of response; plethysmography to measure lung volume; gas transfer evaluation of lung transfer factor for CO (TLCO); imaging by chest X-ray and high-resolution computerized tomography (HRCT) scan; arterial blood gas measurement; and exercise testing.

The cornerstone of management of a patient with COPD is to attempt smoking cessation or reduction. This should be backed by ensuring a healthy diet, adequate exercise, immunization against influenza and bronchodilator therapy (anticholinergic agents and short- and long-acting β_2-agonists), and by considering pulmonary rehabilitation, long-term oxygen therapy and volume-reduction surgery in more severe cases. The World Health Organization and the U.S. National Heart Lung and Blood Institute have organized the Global Obstructive Lung Disease (GOLD) guidelines, which are analogous to the Global Initiative on Asthma (GINA) guidelines and which should be available on the National Institutes of Health website by May 2001.

Recently licensed therapies that are proving effective in the management of COPD include the combination of **ipratropium** and **salbutamol** (*Combivent*, Boehringer Ingelheim) and of the long-acting β_2-agonists **formoterol** and **salmeterol**. Promising therapies in phase III clinical trials include the long-acting anticholinergic (M_1/M_3 antagonist) **tiotropium bromide** (*Spiriva*, Boehringer Ingelheim; Fig. 2), which is employed in a single daily inhaled dose.

Inhaled steroids have been studied in four long-term, three-year studies in COPD (Euroscop, ISOLDE, Copen-

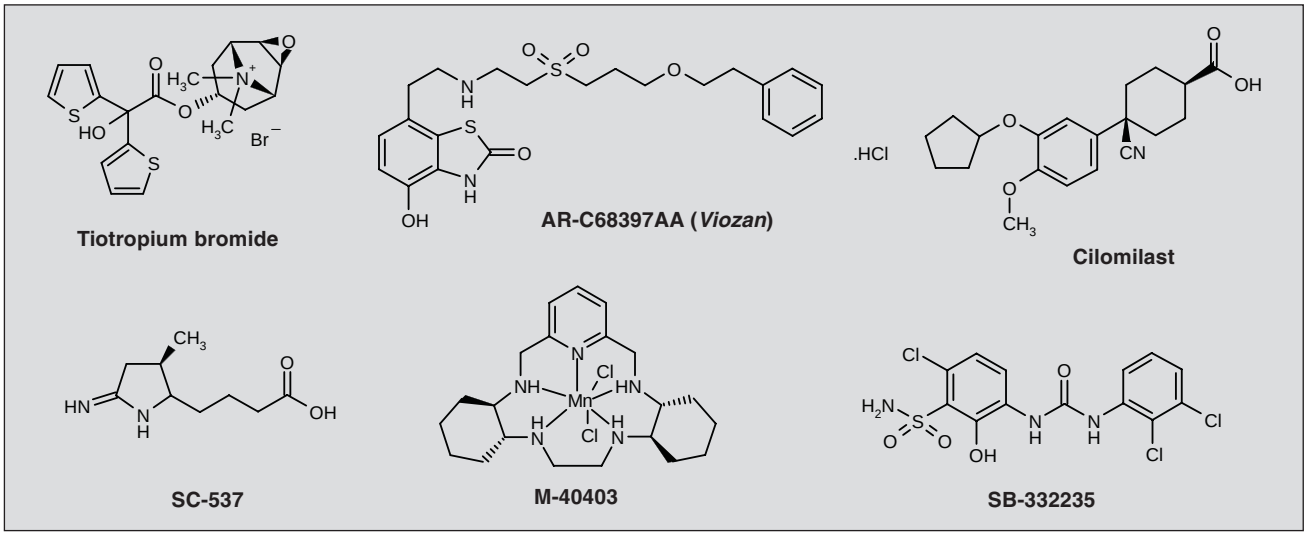

Fig. 2. Structures of selected compounds discussed at the meeting.

hagen, Lung Health 2), and although benefit can be demonstrated in terms of improvement in quality of life, the studies have been disappointing in that they have failed to convincingly alter the progressive fall in FEV_1 that occurs in COPD. Moreover, this expensive high-dose therapy carries a high risk of systemic adverse events in this high-risk elderly smoking population.

Smoking cessation has proved remarkably difficult for most patients with COPD, despite the availability of nicotine replacement therapy and **bupropion hydrochloride** (*Zyban*, GlaxoSmithKline), so that there remains a major need for new therapies for COPD. There is the requirement for specialized antiinflammatory agents that act on neutrophils, macrophages, proteases, cytokines, chemokines and mediators. Since the small airways and alveoli need to be targets, oral therapy may have advantages over the inhaled route. The particular overriding need is to retard or even reverse the loss in lung function in COPD, thus providing genuine disease modification.

Viozan

Representing a novel approach that is supported by preclinical and clinical data in the symptomatic treatment of COPD, **AR-C68397AA** (*Viozan*, AstraZeneca; Fig. 2) is now in phase III studies. *Viozan* is a dual dopamine D_2 receptor agonist and β_2-adrenoceptor agonist being developed to cause bronchodilatation and limit breathlessness, cough and mucus production. Alan Young of AstraZeneca (Charn-wood, U.K.) presented the rationale for dopamine agonism: to activate D_2 receptors on sensory nerve endings, thus inhibiting reflex neuronal mechanisms that contribute to the symptoms of COPD. *Viozan* is a potent β_2-agonist in anesthetized ventilated dogs. In addition, D_2-agonist activity can be studied in anesthetized β-blocked dogs, where there is inhibition of histamine-induced discharge of rapidly adapting receptors, reflex-induced tachypnea and ammonia-induced mucus production. Furthermore, *Viozan* inhibits capsaicin-induced cough in conscious β-blocked dogs.

Three phase II clinical studies of inhaled *Viozan* have now been completed in 2,300 patients treated for 4–6 weeks. Study I involved a proof of principle and a pressurized metered-dose inhaler (pMDI); Study 2, a dose-ranging study of a pMDI; and Study 3, a dose-ranging study of *Turbuhaler*. These studies have demonstrated that *Viozan* causes superior control of symptoms when compared with **salbutamol** or **ipratropium bromide** given three times daily. *Viozan* at the high dose of 495 μg t.i.d. was well tolerated, not causing nausea and vomiting, but a discernible taste was noted in 20% of the subjects.

Cilomilast

The novel oral phosphodiesterase type 4 (PDE 4) inhibitor **cilomilast** (Ariflo, GlaxoSmithKline; Fig. 2) was presented by Chris Compton of GlaxoSmithKline (Harwood, U.K.). Owing to their ability to elevate cAMP content in key target cells, PDE4 inhibitors have antiinflammatory, bronchodilatory and neuromodulatory activities. First-generation PDE4 inhibitors such as rolipram, however, have limiting side effects such as nausea, vomiting and increased gastric acid secretion. Second-generation PDE4 inhibitors have been chosen using a strategy based on selection of antagonism of the low-affinity binding confomer for rolipram that is located on inflammatory leukocytes, while minimizing antagonism of the high-affinity binding confomer for rolipram that is found on central nervous system and gastric parietal cells. This approach maximizes antiinflammatory activity, yet reduces the potential to cause nausea and vomiting. Indeed, preclinical studies demonstrate the activity of cilomilast in inhibiting neutrophil recruitment, chemotaxis, activation and IL-8 production. In a six-week dose-range finding study in COPD patients with a predicted mean

FEV_1 of 57%, cilomilast 15 mg b.i.d. caused a 10% improvement in trough FEV_1. Phase III studies have been performed in Europe and the United States, and preliminary results will be presented at the American Thoracic Society (ATS) meeting in San Francisco in May 2001.

Modulation of nitric oxide, superoxide and peroxynitrite

Mark Currie (Sepracor, Marlborough, Massachusetts, U.S.A.) presented his research on strategies against toxic oxidant species. Selective inhibition of the inducible form of nitric oxide synthase (*selective iNOS inhibitors*) is an attractive therapeutic target in a variety of inflammatory diseases. The constitutive forms of NOS comprise endothelial eNOS, which maintains blood pressure, inhibits platelet aggregation and hinders leukocyte adhesion, and neuronal nNOS, which is a neurotransmitter that promotes gastrointestinal motility. Exhaled nitric oxide (NO) is elevated in the breath of asthmatics, epithelial iNOS is expressed and can be inhibited by steroids, and 3-nitrotyrosine immunoreactivity can be demonstrated. In a Lewis rat lipopolysaccharide (LPS) model, the selective iNOS inhibitor **SC-537** (Fig. 2) causes pronounced inhibition of plasma nitrite, reduction of exhaled NO and inhibition of tissue iNOS, but only causes elevation in blood pressure at higher doses.

Superoxide dismutase (SOD) mimics are small molecules with catalytic activity similar to SOD and include the manganese-containing **M-40403** (MetaPhore; Fig. 2). This molecule inhibits carrageenan-induced changes in rodent paw volume, as well as in lung and intestinal inflammatory models. SOD mimics also exert biochemical protection against norepinephrine breakdown during endotoxic shock.

Peroxynitrite catalysts protect against cell death in the rodent paw model as judged by lactate dehydrogenase release. These molecules scavenge peroxynitrite and convert it to nitrate, resulting in a reduction in the *nitrite:nitrate* ratio in rodent models. Vitamin B_{12a} (hydroxocobalamin) represents a *NO scavenger* that prevents or reverses LPS-induced shock in rodents. Hydroxocobalamin is thought to trap NO and cause its distribution from the vascular compartment into the urine.

Protease inhibition

Matrix-degrading metalloproteases, serine proteases and cysteine proteases are expressed during specific periods of tissue development and repair. Stephen Shapiro (Washington University School of Medicine, St. Louis, Missouri, U.S.A.) described how neutrophils produce neutrophil elastase, cathepsin G and proteinase 3 as well as MMP-8 and -9. In contrast, macrophages have a complement of cathepsin S and L and MMP-1, -3, -7, -9 and -12, as well as MT-MMP-1. Macrophage elastase has been demonstrated to be MMP-12 and is coded on human chromosome 11q. MMP-12 degrades a variety of substrates, including elastin, but not interstitial collagen.

Gene targeting has been useful in directly demonstrating roles for individual MMPs and elastases in tissue destruction. The macrophage metalloelastase (MME) (MMP-12) double-knockout ($^{-/-}$) mouse has markedly diminished capacity of macrophages to degrade extracellular matrix component, such as matrigel. These mice are protected from the development of cigarette smoke-induced pulmonary emphysema and fail to recruit alveolar macrophages in response to cigarette smoke. These mice, however, have compromised host defense, with deficiencies against certain tumors and Gram-positive bacterial infection.

Mice deficient in gelatinase B, MMP-9 double knockouts ($^{-/-}$) are short owing to a failure of primary angiogenesis in the metatarsal growth plate. MMP-9 may function physiologically in long bone development and contact hypersensitivity, while acting pathologically in atherosclerosis, aortic aneurysms and bullous pemphigoid.

Mice knockouts for neutrophil elastase are 60% protected from cigarette smoke-induced emphysema. This suggests interaction between neutrophils and macrophages in the development of emphysema. Furthermore, neutrophil elastase is abundant in the neutrophil primary granules and is important physiologically in host defense against Gram-negative bacteria.

In considering therapies directed at inhibiting proteases for use in COPD, it is important to be aware of the potential to inhibit host defense against particular types of infection and tumors. It should also be considered that macrophage and neutrophil interactions could be fundamental to the pathogenesis of alveolar destruction that occurs in emphysema.

Neutrophil chemokine receptors

Henry (Skip) Sarau of GlaxoSmithKline Pharmaceuticals presented an overview on strategies directed against neutrophil chemokine receptors. The chemoattractant cytokines are small proteins of 70–80 amino acids. The molecular configuration of their four highly conserved cysteines permits classification into four groups: α (C-X-C), β (C-C), γ (C) and δ (C-X-X-X-C). Chemokine receptors have been ascribed to the following members: CCR 1–10, CR1, viral and Duffy receptors, CXCR 1–5 and CX3CR1. The presentation centered on the neutrophil CXCR receptors, which are members of the superfamily of G-protein–coupled, seven transmembrane-spanning receptors. CXCR1 binds IL-8 and GCP-2, while CXCR2 binds IL-8, GRO-α, -β and -γ, NAP-2, ENA-78 and GCP-2. CXCR2 appears to be responsible for IL-8–induced chemotaxis of human neutrophils and T cells, while CXCR1 is responsible for neutrophil degranulation.

SB-332235 (Fig. 2) is a selective CXCR2 antagonist, with an IC_{50} for CXCR1 of 9.6 μM and an IC_{50} for CXR2 of 9.3 nM. This molecule potently inhibits IL-8– and GROα-

induced chemotaxis of human neutrophils, T cells and dendritic cells. The molecule has been studied in an LPS-induced rabbit airways neutrophilia model, an acute arthritis model in New Zealand white rabbits and a phorbol ester (PMA)-induced cutaneous inflammatory model in the rabbit. Interestingly, several recent reports have shown increased amounts of IL-8 in sputum supernatants from patients with COPD. SB-332235 is a promising potential therapy for pulmonary disease (COPD and more severe asthma), rheumatoid arthritis, psoriasis and inflammatory bowel disease.

Monoclonal antibodies

Ted Torphy of Centocor (Malvern, Pennsylvania, U.S.A.) began by describing the development of a range of therapeutic monoclonal antibody (mAb) types that have progressed from being of murine to human origin. The murine anti-CD3 muromonab-CD3 (*Orthoclone*, Ortho Biotech) was launched in 1986; the anti-TNF-α chimeric mAb infliximab (*Remicade*, Centocor) has been licensed for rheumatoid arthritis and Crohn's disease; the anti-HER-2 humanized mAb trastuzumab (*Herceptin*, Roche) employed in cancer therapy, and the fully human anti-TNF-α mAb D2E7 (Cambridge Antibody Technology) is currently in phase III clinical studies.

Compared with traditional low-molecular-weight drugs, mAbs have several attractive features. Firstly, mAbs have a high degree of selectivity for their molecular targets, resulting in predictable biological effects. Secondly, mAbs have fewer side effects since they do not affect hepatic cytochrome P450 drug metabolism pathways and have reduced potential for drug interactions. Thirdly, there are predictable and prolonged pharmacokinetics. Finally, with advanced technical development, R&D cycle times are considerably reduced.

The technology to develop mAbs has advanced considerably in the past decade, moving from hybridoma and chimeric methods to transgenic mice, while bacteriophage display offers the chance to select from an extended library of specificities. In addition, some of the hurdles in development of mAb therapies have been overcome. New delivery technologies are being evaluated to minimize the inconvenience and lack of patient acceptability for injections. New manufacturing technologies based on transgenic animals and plants could reduce production costs by 25-fold. The immunogenicity of mAbs has been reduced by production of humanized or fully human forms.

The range of cells and mediators that have been implicated in the pathogenesis of COPD include TNF-α, IL-6, IL-8 and epidermal growth factor (EGF); therapeutic mAbs have already been generated against these targets. Following the one-year-long ATTRACT study, infliximab has recently been licensed by the FDA as an agent that inhibits the progression of joint damage in rheumatoid arthritis. This offers the insight that COPD, despite being a chronic progressive lung disease, could be amenable to disease modification through mAb therapy.

Retinoids

In the final talk of the day, Paula Belloni of Roche Bioscience (Palo Alto, California, U.S.A.) presented a comprehensive update on the development of retinoic acid receptor agonists, or vitamin A analogues, for COPD. Retinoids have been employed clinically to treat a variety of inflammatory skin disorders, especially to promote the repair of ultraviolet-induced skin damage. Retinoic acid receptor (RAR) agonists have the potential to be used as anabolic therapy to reverse or retard the loss of lung function that occurs in COPD.

Retinoids, natural and synthetic, as a class are structurally related to vitamin A (retinol). They are pleiotropic regulatory compounds that modulate structure and function of cells at various times during and after tissue development. Retinol is primarily stored in the liver as retinyl esters; it is carried in the plasma bound to retinol-binding protein (RBP) and is converted within the cell cytoplasm of target tissues to the active hormone *all-trans*-retinoic acid (ATRA). Retinoids are inactive while bound to the cytoplasmic receptors CRBP and CRABP and exert biological activity via the nuclear retinoic acid receptors. These receptors are ligand-inducible transcription factors belonging to the steroid nuclear hormone receptor superfamily. ATRA-bound heterodimers of RAR/RXR bind to RA-response elements (RARE) to promote and/or suppress gene expression. ATRA has roles in modulating lung structure and function, branching morphogenesis of the embryonic lung, peptide growth factor receptor expression, matrix protein metabolism and mucociliary function. The major role in relation to COPD therapy, however, is alveolar type II epithelial cell and myofibroblast proliferation, differentiation and elastin synthesis, resulting in alveolar septation.

Retinoids and RARs are temporally and spatially regulated during lung development and drive branching through regulation of homeobox genes. The last phase of branching, alveolar septation, is regulated by RARγ and increased local concentrations of ATRA within and interstitial myofibroblast. Bronchopulmonary dysplasia in infancy is associated with a retinol deficiency, delayed alveolar septation and impaired lung function. Retinol deficiency is also associated with COPD patients, and preclinical studies suggest that a key carcinogen in cigarette smoke, benzpyrene, induces local vitamin A deficiency in the lung. Since retinoids can inhibit matrix metalloprotease transcription through activator protein-1 (AP-1), local deficiency in ATRA may contribute to the protease/antiprotease imbalance associated with COPD.

In an important series of experiments published in 1997, Don and Gloria Massaro described the role of ATRA in promoting alveolar septation

in the rat, noting that RARβ impairs septation. Recently, McGowan et al. noted that in RARγ ($^{-/-}$) knockout mice, there is reduced lung elastin and alveolar septation. **Ro-44-4753** is a selective RARγ agonist that drives tropo-elastin gene expression and alveolar repair and/or alveolarization in adult rats. Ro-44-4753 has been shown to induce alveolar repair in two rodent models, pancreatic elastase-induced emphysema in rats, and cigarette smoke-induced emphysema in mice. Lung function assessed by PaO_2 is also improved by treatment with ATRA or Ro-44-4753 in elastase-injured rats. Tepper et al. (2000) have also shown that ATRA partially reverses lung function in elastase-damaged rats, enabling increased lung volume and compliance and a decrease in carbon monoxide diffusion capacity (DLCO).

The critical question is whether the adult human lung is capable of alveolar repair, since adult human lungs may not have the capacity to regenerate alveoli. It is, however, encouraging that recovery from adult respiratory distress syndrome can occur. In COPD an inverse relationship has always been found between plasma retinol status and the degree of airway obstruction, while a small study has demonstrated that therapy with retinol palmitate improves FEV_1 in COPD. It has been hypothesized that high levels of RAR activity may be associated with chronic repair and the mucous hyperplasia of chronic bronchitis, while low levels of RAR activity may be associated with inadequate repair of damaged elastin resulting in emphysema.

ATRA, **isotretinoin** (*Roaccutane*, 13-*cis*-RA, Roche) and 9-*cis*-RA are RAR pan-agonists that have a narrow therapeutic window, mainly owing to teratogenicity and hepatotoxicity. Teratogenicity is believed to be a factor for all retinoids by virtue of their role in pattern formation via homeobox gene activity. Other adverse events include psychiatric disorders, hypertension, elevated blood lipids (triglyceride and cholesterol), hyperostosis and mucus hypersecretion.

Selective RARγ agonists—such as **Ro-44-4753**—have a range of advantages over ATRA, in particular a lack of hepatotoxicity because the liver does not have RARγ receptors. There are no apparent effects on triglyceride levels (experimental therapeutic window ≥ 3000). In addition mucus hypersecretion does not occur; however, the problem of teratogenicity remains (albeit less of an issue in elderly females), and mucocutaneous effects of dry skin and chapped lips may occur.

A group of investigators from Los Angeles has undertaken a proof of concept study with ATRA in COPD (Mao, ATS, 2000). Twenty COPD patients were enrolled in a six-month, two-way crossover study, administering ATRA 25 mg/m^2 for three months. ATRA was generally well tolerated, with the anticipated side effects noted. No significant changes in lung function occurred; however, preliminary assessment of HRCT images suggests improvement in a subset of structural measures, and 8 of 20 patients reported positive subjective responses.

The National Institutes of Health is also undertaking a study of retinoid therapy in COPD called FORTE (Feasibility Of Retinoid Therapy for Emphysema). In the multicenter study, 300 COPD patients will be randomized into one of four treatment arms, placebo, 13-*cis*-RA, low-dose ATRA and high-dose ATRA. Treatment outcome measures include HRCT to assess lung structure, lung-function testing, quality of life and bronchoscopy to explore potential surrogate markers associated with retinoid-induced repair.

A number of issues remain to be resolved for the therapeutic use of RAR-selective agonists in COPD. The relative merits of pan agonists compared with selective agonists need to be considered, the route of delivery (oral or inhaled) needs to be defined, and proof of concept clinical studies need to be performed. Clinical studies may need to be performed in current and former cigarette smokers, both early and late in the progression of COPD.

Conclusion

This meeting ended on a note of cautious optimism, in that progress is certainly being made with the various therapeutic strategies covered in the course of the symposium. **Tiotropium bromide** and **AR-C68397AA** are likely to be licensed in the near future and will offer symptomatic benefits to COPD patients, while the results of large scale studies of **cilomilast** on lung function are awaited with great interest. Therapies to combat reactive oxidant species, neutrophils and proteases are entering early clinical studies, while retinoids offer perhaps the greatest potential for lung repair and regeneration. There remains, however, the requirement to improve our knowledge of the molecular pathogenesis of COPD, to develop noninvasive techniques for the classification and monitoring of this disease and to develop reliable clinical trial methodology. The inspiration to these efforts originates from the lack of effective therapy for this disease of growing magnitude in our society.

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