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REVIEW ARTICLE

CHEMOKINE RECEPTOR CXCR2 ANTAGONISM TO PREVENT AIRWAYS INFLAMMATION

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SUMMARY

Neutrophilic inflammation is associated with several pulmonary conditions, including chronic obstructive pulmonary disease (COPD), severe asthma and cystic fibrosis, and contributes to disease pathophysiology. The underlying inflammation in each of these diseases is resistant to glucocorticosteroid therapy and is therefore persistent and difficult to treat. The chemokine receptor CXCR2 is highly expressed on the surface of neutrophils and has been targeted by a number of pharmaceutical companies as a potential antiinflammatory therapy. CXCR2 receptor antagonists have been shown to inhibit neutrophil accumulation in a number of preclinical models of pulmonary inflammation and show good selectivity in human cells ex vivo. Few CXCR2 receptor antagonists have been reported in clinical trials, although SCH-527123 and SB-656933 have both been shown to be effective in reducing ozone-induced airways neutrophilia in healthy volunteers. Studies are now under way in patients with COPD and preliminary data have indicated efficacy for SCH-527123. Further studies on the effects of CXCR2 receptor antagonists are required; however, they appear to be promising, novel antiinflammatory therapies for a number of pulmonary inflammatory diseases.

BACKGROUND

The migration of leukocytes to the site of an inflammatory insult is regulated by the expression of chemotactic factors. These include chemokines, chemotactic cytokines that can be released from a number of inflammatory and structural cells following stimulation. Chemokines are small cytokines, typically 8-10 kDa containing four conserved cysteine residues linked by disulfide bonds (1). They are subclassified into four groups, namely CXC, CC, C and CX_3C according to the structure and spacing of these conserved cysteine residues. To date, in humans, there are over 50 known chemokines, but only 19 receptors have been discovered (2, 3), indicating a degree of redundancy. All chemokine receptors belong to the superfamily of seven-transmembrane G protein-coupled receptors (GPCRs). Classically, GPCRs are highly amenable to inhibition by pharmacological agents (4), and for this reason, there has been much activity within the pharmaceutical industry to develop small-molecule antagonists against these receptors for use as antiinflammatory agents in a number of conditions, including airways inflammation (5, 6).

CXCR2 was first cloned in 1991 and identified as an interleukin-8-binding protein that contains 360 amino acids, with about 77% similarity with CXCR1 (7). CXCR2 is highly expressed on the surface of neutrophils and is considered a regulator of neutrophilic inflammation (8, 9). Activation of CXCR2 occurs via heterotrimeric G-proteins, comprising Gα, Gβ and Gγ. CXCR2 can associate with a number of Gα subunits, both pertussis-sensitive and -insensitive, leading to activation of distinct intracellular pathways controlling the release of intracellular calcium and activation of phosphatidylinositol 3-kinase (PI3K), leading to cell migration (10). CXCR2 is also expressed on approximately 45% of circulating monocytes (11) and 5-25% of circulating T lymphocytes (12), suggesting that this receptor may regulate a number of leukocytes involved in the inflammatory response. CXCR2 is a promiscuous receptor that binds a number of CXC chemokines, including CXCL1, CXCL2, CXCL3, CXCL5, CXCL7 and CXCL8 (Fig. 1). In addition to this promiscuity, CXCR2 is often co-expressed with CXCR1, which also binds and is activated by CXCL8 with similar affinity (13), although the function of these two receptors may differ with respect to neutrophil migration and degranulation. However, the lack of selective CXCR1 antagonists has made definitive studies difficult. Nevertheless, CXCR2 is less selective with respect to ligand binding compared to CXCR1, but the biological relevance of coexpression of these receptors has yet to be determined, although this has been reviewed in depth recently and will not be addressed further in the current article.

Recently, a new ligand for the CXCR2 receptor has been identified (14). Breakdown of the extracellular matrix and tissue remodeling is a feature of many pulmonary diseases, including chronic obstructive pulmonary disease (COPD) (15-17) and asthma (18-20). Breakdown of the extracellular matrix produces a number of peptide fragments,

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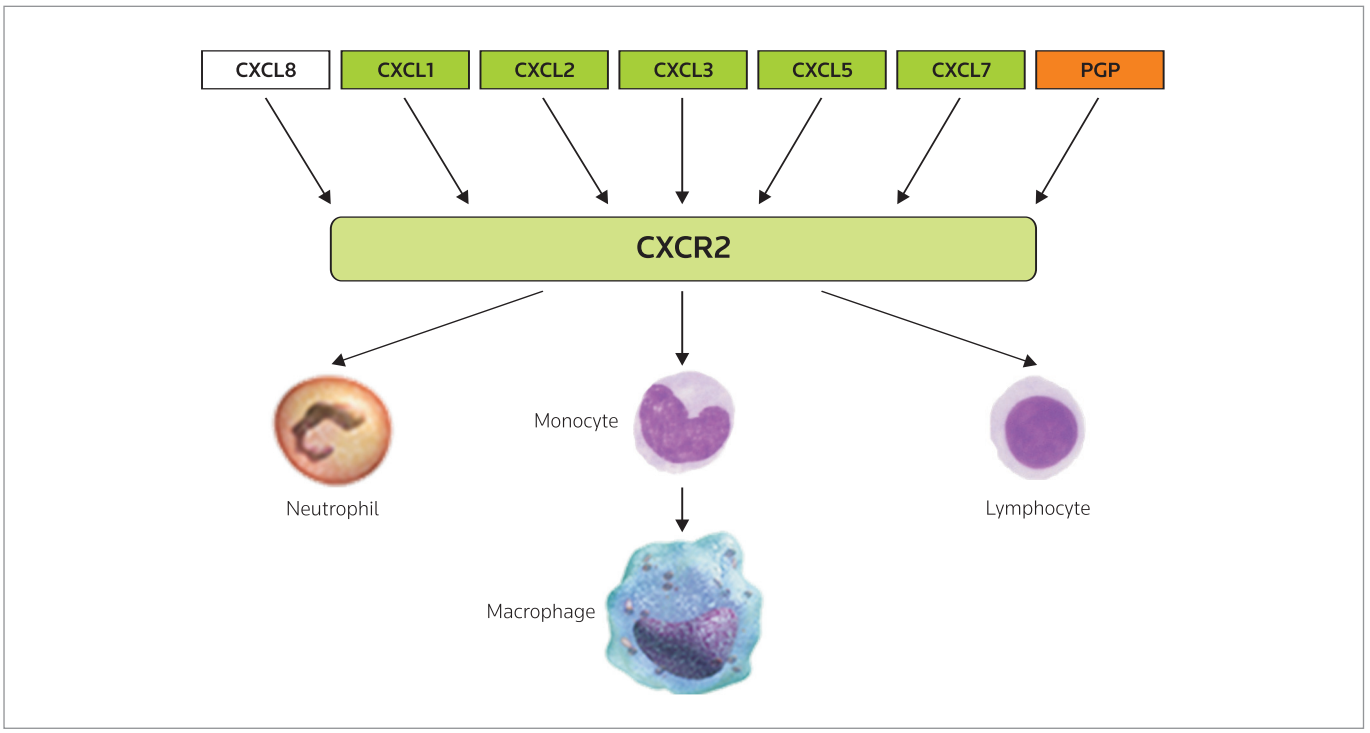

Figure 1. Ligands and cells expressing the CXCR2 receptor.

including *N*-acetyl-Pro-Gly-Pro (PGP), which signals through activation of CXCR2 and acts to recruit neutrophils and generate the production of superoxide radicals (14). There is some controversy in the literature as to whether *N*-acetyl-PGP binds directly at CXCR2 (21), although more recent studies suggest that *N*-acetyl-PGP could contribute to the recruitment of neutrophils observed in destructive pulmonary diseases such as COPD (22-25).

From these data, neutrophilic inflammation appears to be largely regulated through CXCR2 (8, 9). There are a number of inflammatory lung diseases where neutrophilia is a key feature, and this influx of inflammatory cells contributes to the underlying pathophysiology. These diseases include COPD, severe asthma and cystic fibrosis, where current therapies are limited and purely symptomatic. Alternative approaches are needed for the treatment of neutrophilic inflammatory diseases and targeting CXCR2 may prove to be a therapeutic option (26).

COPD

COPD is an increasing global health problem and is predicted to become the third largest cause of death by 2020 (27). The term COPD encompasses three underlying pathophysiologies: chronic bronchitis or mucus hypersecretion, small airways disease (bronchiolitis) and emphysema (28, 29). Among the industrialized nations, the major risk factor for COPD is cigarette smoking (30, 31). To date, smoking cessation is the only intervention that prevents the onset of disease, although once established, smoking cessation does not alter the underlying inflammatory process observed in the lungs of these patients (32).

Pulmonary inflammation in COPD is associated with increased leukocyte infiltration comprising macrophages, neutrophils and $CD8^+$ T lymphocytes (28). Recruitment of neutrophils to the airways in COPD is thought to be driven by increased production of CXC chemokines, and levels of CXCL1, CXCL5, CXCL7 and CXCL8 have all been shown to be elevated in induced sputum of these patients. The expression of CXCL7 in the bronchial mucosa of severe, stable COPD patients is also elevated and correlates with increased neutrophil activation, which could contribute to the pathophysiology of this disease (33). Since CXCR2 is also expressed by monocytes (11), these chemokines may also be responsible for the increased macrophage load found in COPD.

Chemotaxis of both neutrophils and monocytes appears to be altered in COPD. Early chemotaxis studies showed that neutrophils from COPD patients migrated more towards chemotactic stimuli than cells from control subjects (34). More recently, neutrophils from patients with COPD have been shown to display aberrant migration towards a number of chemoattractants, including CXCL1 and CXCL8. COPD neutrophils appear to move more rapidly but are less directed when compared to cells from nonsmokers and smokers (35). This is not associated with any differences in cell surface receptor expression, but appears to be due to alteration in cell signaling pathways. Monocytes from patients with COPD are also abnormal in their migratory response to CXCL1 and CXCL7. Under these conditions, ligation of CXCR2 with specific ligands drives an enhanced migratory response compared with monocytes from smokers and nonsmokers, due to an alteration in CXCR2 recycling to the cell surface and not to global expression (11). Taken together, these aberrant

responses of both neutrophils and monocytes to migratory signals through the CXCR2 receptor are likely to contribute towards the abnormal inflammatory pulmonary infiltration observed in COPD. Once in the lung, macrophages and neutrophils can modulate all the pathophysiological features of the disease; therefore, preventing the migration of neutrophils and monocytes from the circulation could have therapeutic benefit in COPD.

Asthma

Asthma is a highly prevalent disease associated with reversible airflow obstruction and airways hyperresponsiveness. As with COPD, asthma is considered an inflammatory lung disease with the pulmonary infiltrate comprising eosinophils and Th2 lymphocytes (36-38). Asthma is well controlled with a number of pharmacological agents, such as bronchodilators and antiinflammatory agents (39). However, control of symptoms is not possible in about 5% of patients (39, 40). These severe asthmatics are resistant to glucocorticosteroid treatment, which is associated with increased neutrophilia (40). Increased neutrophil numbers are also found in the airways of asthmatics during an exacerbation and may be associated with increased levels of CXCL8 due to viral infection (41). In addition, neutrophilia is also observed in the airways of smoking asthmatics (42). The role of the neutrophil in asthma pathology is not clear, but it has been hypothesized that neutrophil elastase may degrade mucus plugs and may therefore have some beneficial role in severe asthma (43). Nevertheless, neutrophils are associated with more severe asthma and airways narrowing (44, 45), suggesting that targeting this leukocyte infiltration may have some benefit in this group of asthmatic patients (46).

Cystic fibrosis

Cystic fibrosis (CF) is an autosomal recessive disorder that leads to a defective epithelial chloride channel (47). Patients with CF exhibit numerous infectious episodes that are associated with neutrophilic inflammation. Pharmacotherapy for CF is currently inadequate, with glucocorticosteroids having little effect on the underlying inflammation. Neutrophil numbers are elevated in the induced sputum of patients with CF (48) and this is associated with increased levels of chemokines, including CXCL8, which may drive neutrophilia via CXCR2. Neutralization of CXCL8 did not reduce the chemotactic capacity of CF sputum for neutrophil migration; however, rather than blockade of a single chemokine, targeting a receptor directory might prove to be a more effective antiinflammatory therapy.

Acute lung injury

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are associated with an acute inflammation characterized by neutrophilic infiltration into the alveolar compartments (49). Mortality from ALI/ARDS remains high despite improvements in intensive care medicine (50). Elevated concentrations of CXCL1 and CXCL8 are found in the bronchoalveolar lavage (BAL) fluid of patients with ARDS (51-54) and may drive the observed neutrophilic inflammation. Further support that the CXCR2 axis may be critical in driving the pathology of ARDS has come from a mouse model of ventilator-induced lung injury, whereby neutralizing antibodies against CXCL1 were able to attenuate the neutrophilic response, with further confirmation being derived from CXCR2 knockout mice (55). While there are no studies examining the effect of a CXCR2 receptor antagonist in ARDS, the contribution of neutrophils to ALI and ARDS suggests that a pharmaceutical approach to blockade of CXCR2 could prove to be beneficial in these patients.

Idiopathic pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) is associated with deposition of extracellular matrix proteins, leading to fibrosis of the lung, which is detected by computed tomography (CT). The mechanisms of fibroblast proliferation are not clearly understood, although angiogenesis is a feature of these fibrotic lesions (56). CXC chemokines that contain an ELR motif exhibit angiogenic properties (57-59), with many of these, including CXCL5 and CXCL8, being ligands at CXCR2. BAL fluid and serum of patients with IPF contain elevated concentrations of CXCR2 receptor ligands (60, 61) together with increased tissue expression (58, 59), and there is a marked sputum neutrophilia associated with increased concentrations of CXCL8 (62). Taken together, these data provide supporting evidence of a role for CXCR2 in IPF. Exactly how CXCR2 may mediate these effects is unclear, but CXCR2 may be expressed on the surface of structural cells, including endothelial cells and fibroblasts (63, 64), and may therefore have a role to play in driving the fibrotic process under disease conditions. Indeed, neovascularization of the mouse lung is reduced in CXCR2 knockout mice following left pulmonary artery ligation (65). Therefore, CXCR2 receptor antagonists may prove to have efficacy in ameliorating the inflammatory response in these patients and may even impact upon lung remodeling.

Several pharmaceutical companies have developed chemical entities that are antagonists at the CXCR2 receptor (Fig. 2). Many of these have progressed through in vitro studies and are currently undergoing clinical trials for use in neutrophilic inflammatory diseases. However, to date, there are no CXCR2 receptor antagonists approved for use in humans, although several are presently undergoing clinical trials.

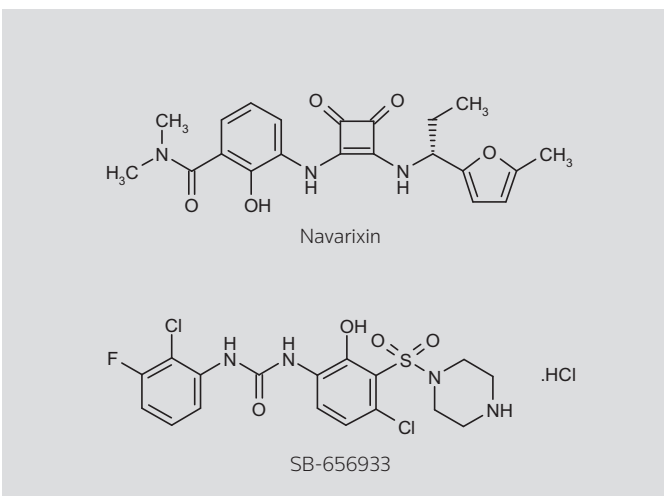

Figure 2. Structures of CXCR2 receptor antagonists.

PRECLINICAL PHARMACOLOGY

The CXCR2 receptor antagonists currently under investigation fall into four related entities: *N,N'*-diphenylureas, nicotinamide *N*-oxides, quinoxalines and triazolethiols. The first nonpeptide CXCR2 receptor antagonist reported was SB-225002, which was identified using high-throughput screening and inhibited both human and rabbit neutrophil migration in vitro and neutrophil migration in rabbits (66). Following on from this, GlaxoSmithKline has developed several additional molecules that are selective CXCR2 receptor antagonists (67). These include SB-455821, SB-265610, SB-332235 and SB-656933 (9, 68). Early preclinical pharmacology testing using rabbit models of neutrophilic inflammation demonstrated antiinflammatory effects for SB-265610 (69). The efficacy of this molecule was also confirmed in hyperoxia-mediated lung neutrophilia in rats (70). Subsequently, SB-455821 was shown to inhibit C-X-C motif chemokine 2 (macrophage inflammatory protein 2, MIP2)-dependent neutrophil migration in mice (68) and SB-332235 blocked cigarette smoke-induced neutrophilia in rats (71). Similarly, both SB-225002 and SB-332235 inhibited PGP-mediated pulmonary neutrophilia in mice (22). Taken together, these studies provide support for the role of CXCR2 in neutrophilic inflammation.

Neutrophil migration into the lung is commonly associated with infection. SB-225002 has been shown to be effective in reducing neutrophil influx in a murine model of pulmonary *Streptococcus pneumoniae* infection. In addition, this molecule also attenuated macrophage recruitment, thereby confirming a role for CXCR2 in this response (72). More detailed pharmacological analysis of SB-265610 has shown that this molecule acts as an allosteric inverse agonist at CXCR2 receptors and appears to interfere with coupling of the receptor to G-proteins, hence inhibiting downstream signaling (73).

These molecules have also been investigated in various assays in human cells from both healthy individuals and patients with pulmonary diseases. An early analogue of SB-225002, SB-272844, was shown to inhibit shape changes of human neutrophils in response to CXCL1, with a K_d of 123 ± 18 nM (74), and both CXCL1- and CXCL8-mediated inhibition of neutrophil apoptosis, with K_d values of 49.9 and 253 nM, respectively (75). SB-332235 blocks monocyte migration towards CXCL1, CXCL5 and CXCL7, but was less effective against CXCL8. Of note, SB-468477, a combined CXCR1 and CXCR2 receptor antagonist, was more effective in these cells (11). SB-656933 is the current clinical lead molecule from GlaxoSmithKline and is undergoing clinical trials. In ex vivo studies, this molecule inhibited CXCL1-mediated shape change and CD11b upregulation in neutrophils from patients with COPD, with IC_{50} values of 310.5 and 260.7 nM, respectively (76).

Schering-Plough (Merck & Co.) has described a potent, orally active CXCR2 receptor antagonist, navarixin (SCH-527123), that also has activity at the CXCR1 receptor (77). Navarixin blocked neutrophil accumulation in both mouse and rat models of pulmonary inflammation induced by intratracheal administration of lipopolysaccharide (78). In addition, this molecule also inhibited mucus production and goblet cell hyperplasia in this model. Using human neutrophils ex vivo, navarixin was characterized further and was shown to be an allosteric inhibitor at the CXCR2 receptor (79), with a K_d of 0.049 ± 0.004 nM compared with a K_d of 3.9 ± 0.3 nM at the CXCR1 receptor, and could inhibit CXCL8-mediated human neutrophil migration and release of myeloperoxidase (77, 79).

AstraZeneca has also had a number of programs examining the synthesis and development of CXCR2 receptor antagonists based on both triazolethiols (80) and thiazolopyrimidines (81), although no preclinical pharmacology has been reported. One candidate molecule, AZD-8309, has been investigated in a clinical trial, although development of this compound has been discontinued and the company has not disclosed the reasons. More recently, AstraZeneca reported a series of bicyclic CXCR2 receptor antagonists with additional effects at the CCR2 receptor (82), which may have additional antiinflammatory effects that could be beneficial in pulmonary inflammation.

Other pharmaceutical companies have reported the synthesis of CXCR2 receptor antagonists, including Dompé farmaceutici, which reported a CXCR1/CXCR2 receptor antagonist, repertaxin (also known as reparixin), which has activity against CXCL8-mediated polymorphonuclear leukocyte chemotaxis, with an IC_{50} of about 1 nM (83). The company has also developed a series of antagonists to compete against CXCL8 binding (84) and has reported that DF-2162 inhibited pulmonary neutrophil (PMN) influx in a murine model of bleomycin-induced fibrosis (85) and CXCL1- and CXCL8-mediated chemotaxis of human PMNs at 10 and 1 nM, respectively (86). Pharmacopeia Drug Discovery has also reported the synthesis of imidazolylpyrimidine-based CXCR2 receptor antagonists, but no data are available for human studies (87).

Recently, novel antagonists of the CXCR2 receptor have been developed which are chiral isomers of *N*-acetyl-PGP that block both CXCL8 and *N*-acetyl-PGP by binding to CXCR1 and CXCR2 (88).

CLINICAL STUDIES

Few CXCR2 receptor antagonists have progressed to clinical trials; however, some data are available on three molecules. Schering-Plough/Merck has reported on the effect of a CXCR2 receptor antagonist in man (89-91). Single oral doses of navarixin (10-150 mg) decreased circulating PMNs measured after 24 hours (89), with no adverse events. Subsequent multiple doses of 10 or 50 mg daily for 14 days also showed a decrease in circulating PMNs (90). A study of navarixin given at doses of 3, 10 and 30 mg daily over 12 weeks in patients with COPD showed a mean 20% reduction in circulating neutrophils, although in some patients the fall in neutrophils was much greater (72).

A randomized, double-blind, multiple-dose, placebo-controlled, 3-way crossover trial was performed to examine the effect of navarixin (50 mg) compared with prednisolone (50 mg) on ozone-induced airways neutrophilia in healthy volunteers. Navarixin significantly and almost completely blocked the ozone-induced increase in sputum neutrophils compared with both placebo and prednisolone (91). This trial clearly demonstrated that blockade of CXCR2 could provide a mechanism to reduce airways neutrophilia in man. Schering-Plough/Merck has now examined this antagonist in patients with COPD in a double-blind, placebo-controlled, dose-escalation study with doses of 3, 10 or 30 mg (92). Patients who had received 30 mg of navarixin for 12 weeks had significantly reduced sputum neu-

trophilia (47%), a reduction in matrix metalloproteinase MMP-9 and a significant increase in FEV_1 of 91 mL. This was the first study to show beneficial effects in patients with COPD. Navarixin has also been administered to severe neutrophilic asthmatic subjects in a randomized, double-blind, parallel-group study in 34 patients (93). These patients exhibited > 40% sputum neutrophilia and were given 30 mg of navarixin once a day for 4 weeks. There was a 57% reduction in sputum neutrophilia and there were fewer mild exacerbations in patients on the active drug. There were no changes in FEV_1, but again, there was a decline in blood neutrophils.

AstraZeneca has reported on the effects of AZD-8309 in reducing sputum neutrophilia in healthy volunteers following challenge with inhaled lipopolysaccharide (LPS) (94). AZD-8309 reduced sputum neutrophilia by 79% and significantly reduced neutrophil elastase activity compared with placebo, with no serious or significant adverse effects reported, although AstraZeneca is no longer pursuing this antagonist. A second compound, AZD-5069 is currently undergoing clinical trials in healthy volunteers and patients with COPD and bronchiectasis, although no data on this molecule have yet been reported.

Recently, the first study in humans was also reported by GlaxoSmithKline for SB-656933 (96). Single oral doses (2-1100 mg) of SB-656933 were reported to be well tolerated, with the most frequently observed adverse event being headache (95). The time to maximum plasma concentrations was 2-3 hours and the terminal half-life was reported to be 14-20 hours. Ex vivo, neutrophils also showed a significant reduction in CXCL1-induced upregulation of CD11b. After establishing a safety profile for SB-656933, the efficacy of the drug in attenuating ozone-induced airways neutrophilia was examined. Healthy subjects were exposed to ozone following either 50 or 150 mg SB-656933 and sputum was collected 6 hours later. This study showed that single doses of either 50 or 150 mg of SB-656933 significantly reduced ozone-induced sputum neutrophilia by about 55% and 75%, respectively, thus demonstrating that this molecule was effective at inhibiting neutrophil migration. GlaxoSmithKline currently has a second CXCR2 receptor antagonist, GSK-1325756, undergoing trials in healthy volunteers, but at present no data are available.

SAFETY

There are little safety data reported on the use of CXCR2 receptor antagonists. From the data currently available, oral administration of these molecules has been safe, with no reported adverse events. The major issue with these drugs may be a reduction in circulating neutrophils to a level that may make patients susceptible to infection. To date, both navarixin and SB-656933 have demonstrated a decrease in peripheral blood neutrophils in healthy volunteers, but the clinical significance of this has yet to be investigated.

The possibility of an increased propensity to develop infection due to a reduction in circulating neutrophils may be an issue with the development of CXCR2 receptor antagonists. This is a common risk associated with many antiinflammatory treatments currently under development. This can be highlighted in studies of anti-TNF-α therapy for COPD, where there was an increased incidence of pneumonia (96), and analysis of the Towards a Revolution in COPD Health (TORCH) study, where a combination of salmeterol and fluticasone propionate appeared to increase the risk of pneumonia (97).

FUTURE DEVELOPMENTS

The lack of antiinflammatory therapies for neutrophilic pulmonary diseases such as COPD and severe asthma needs to be addressed. CXCR2 receptor antagonists are exceptionally promising as orally active agents to reduce airways neutrophilia in these diseases. These molecules have the potential to be of benefit in these hitherto difficult to control diseases. The results of the clinical trials that are currently under way are eagerly awaited, although the limited clinical data currently available would suggest that CXCR2 receptor antagonists are beneficial for the treatment of pulmonary inflammation.

DISCLOSURES

P.J. Barnes has served on the Scientific Advisory Boards of AstraZeneca, Boehringer-Ingelheim, Chiesi, GlaxoSmithKline, Novartis, Nycomed, Pfizer, Teva and UCB, and has received research funding from AstraZeneca, Boehringer-Ingelheim, Chiesi, Daiichi Sankyo, GlaxoSmithKline, Novartis, Nycomed and Pfizer. He is also a cofounder of RespiVert (now part of Johnson & Johnson), which has discovered novel inhaled antiinflammatory treatments for asthma and COPD.

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