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Dual dopamine D_2 receptor and β_2-adrenoceptor agonists for the treatment of chronic obstructive pulmonary disease: the pre-clinical rationale

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Abstract This paper describes the rationale for the development of dual dopamine D_2-receptor and β_2-adrenoceptor agonists as potential treatments for the symptoms of chronic obstructive pulmonary disease (COPD). The putative involvement of pulmonary sensory afferent nerves in mediating the key COPD symptoms of breathlessness, cough and excess sputum production is outlined and the hypothesis that activation of D_2-receptors on such nerves would modulate their activity is developed. This premise was tested, in a range of animal models, using the first of a novel class of dual dopamine D_2-receptor and β_2-adrenoceptor agonists, sibenadet HCl (Viozan™, AR-C68397AA). In the course of these studies it was demonstrated that sibenadet, through activation of D_2-receptors, inhibited discharge of rapidly adapting receptors and was effective in reducing reflex-induced tachypnoea, mucus production and cough in the dog. Sibenadet, through its activation of β_2-adrenoceptors, was also shown to be an effective bronchodilator with a prolonged duration of action following topical administration to the lungs. These studies also indicated that sibenadet had a wide therapeutic ratio with respect to expected undesirable side-effects such as emesis and cardiovascular disturbances. These results provided a compelling rationale for the initiation of a clinical development programme with sibenadet for the treatment of COPD.

Keywords chronic obstructive pulmonary disease (COPD); pulmonary sensory nerves; dopamine D_2-receptors; β_2-adrenoceptors; sibenadet HCl (Viozan™, AR-C68397AA)

INTRODUCTION

Pulmonary sensory nerves and their role in the pathophysiology of COPD

Chronic obstructive pulmonary disease (COPD) is a growing worldwide public health issue and is predicted to become the third biggest cause of global mortality by 2020 (1). It is, however, poorly treated (2) as a result of inadequate and limited treatment options. New therapies that reduce the symptoms of breathlessness, cough and excess sputum production, which are characteristic of this disease, would significantly improve the quality of life of patients. All these symptoms can potentially be mediated by neuronal mechanisms in that sensory afferent nerves, activated by endogenous and exogenous irritants, can generate reflexes eliciting cough, mucus production, bronchoconstriction and changes in the depth of breathing (3). This leads to the hypothesis that modulation of sensory nerve activity would have therapeutic benefit in the treatment of lung diseases such as COPD.

Afferent sensory information from the lung is mainly carried within the vagus nerve, the cell bodies of the fibres being found in either of two ganglia, the nodose and jugular located along the cervical vagus. The cell bodies of spinal afferent neurones innervating the airways are situated along the spinal cord. The importance of this sensory innervation is highlighted by findings such as those of Agostini *et al.* (4) who demonstrated that the majority of vagal fibres (approximately 80%) innervating cat bronchi are afferent in nature. Three major classes of sensory nerve fibres have been identified within the airways. Slowly adapting or 'stretch' receptors are associated with reflex control of breathing. These are myelinated afferent fibres located primarily in the smooth muscle, which elicit a long-lasting discharge of action potentials in response to, for example, prolonged and maintained lung inflation. They

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are found throughout the lung, but are primarily concentrated in the large conducting airways. In the dog, approximately 50% of such receptors are thought to be located in the trachea (5). Rapidly adapting (RARs) or 'irritant' receptors are myelinated afferent fibres that are sensitive to a variety of stimuli including chemical irritants such as histamine, as well as mechanical and osmotic stimuli (6). These are located along the length of the entire tracheobronchial tree, but again are found primarily in the larger airways (7). Activation of these RARs is thought to elicit defensive reflexes such as cough, mucus production and rapid shallow breathing (8). In the dog, cigarette smoke has also been shown to stimulate discharge from RARs (9).

Finally, there are C-fibres, which have a high density in the airways and are thought to have a key role in the defence of the lower airways (10). These unmyelinated fibres can be further divided into two groups based upon their anatomical location, these being pulmonary C-fibres found in the peripheral airways and bronchial C-fibres found in the larger airways. Stimulation of C-fibres in dogs leads to a variety of defensive reflexes including cough, tachypnoea (11), mucus secretion (12), apnoea and bronchoconstriction (10). Many C-fibres also contain a variety of neuropeptides in their peripheral processes and may therefore also cause axonal or local reflexes (13).

Dopamine as a neuromodulator of pulmonary sensory nerves

The dopaminergic system in the brain and its involvement in the pathophysiology of neurological and psychiatric disorders of the central nervous system has been the focus of a large body of research. In contrast the role of dopamine receptors in the peripheral nervous system has been less extensively studied. Nevertheless, a number of studies have indicated that dopamine has a role as a peripheral neurotransmitter and that it can modulate the activity of sensory nerves. For example, in anaesthetized cats, dopamine decreases the rate of nerve discharge from the carotid body chemoreceptors (14).

Such findings suggest that dopamine could act to modulate the activity of sensory nerves in the lung. Indeed, dopamine receptor agonists and antagonists have been shown to have a number of effects on lung function in clinical studies. Inhalation of dopamine has been shown to inhibit histamine-induced bronchoconstriction in normal and asthmatic subjects (15), although it did not affect resting bronchomotor tone (16,17). In addition, inhalation of dopamine by asthmatic patients during an exacerbation induces bronchodilatation (17).

There are also several case reports that the dopamine precursor levodopa can improve chronic bronchitis (18) and that the anti-psychotic haloperidol (a dopamine receptor antagonist) can produce bronchospasm (19). The interpretation of such studies is, however, complicated by the fact that dopamine (and the other agents used) has activity at a number of other receptors including both α- and β-adrenoceptors. Nevertheless, an effect of dopamine on dopamine receptors cannot be totally excluded in these studies.

Some of the strongest functional evidence that dopamine can modulate the activity of peripheral sensory nerves in the lung comes from studies in the dog. Jackson and Simpson (20) demonstrated that, in dogs pre-treated with propranolol and phentolamine (to block β- and α-adrenoceptor effects respectively), dopamine infusion inhibited the ability of histamine to stimulate RARs. This inhibition by dopamine was antagonized in animals treated with the selective D_2-receptor antagonist, sulpiride. Dopamine did not affect lung function, as measured by changes in resistance or compliance, suggesting that it was acting directly on sensory nerves rather than by preventing bronchoconstriction, which would activate RARs by mechanical distortion. These observations thus provided direct evidence that dopamine is able to modulate the activity of sensory nerves. The premise that these effects were mediated by dopamine receptors of the D_2-receptor subtype was strengthened by a number of reports in the literature indicating that D_2-receptors (messenger RNA and/or the protein) are present in sensory ganglia (21–26,28).

More recently it has been shown that selective D_2-receptor agonists can modulate the activity of sensory nerves both *in vitro* (27,28) and *in vivo* (29). In the *in vitro* studies, the selective D_2-receptor agonist ropinirole was shown to inhibit the release of the neuropeptide calcitonin gene-related peptide from slices of dorsal spinal cord and airways of the guinea-pig following stimulation with 80 mM potassium chloride (27). The same agonist was also shown to inhibit mobilization of intracellular calcium in rat dorsal root ganglion cells following electrical stimulation (28). In both of these studies, the effects of ropinirole were blocked by sulpiride. The *in vivo* studies demonstrated that another selective D_2-receptor agonist, quinpirole, inhibited prostaglandin E_2-induced hyperresponsiveness of pulmonary C-fibres and ozone-induced tachypnoea in the rat (29). These effects of quinpirole were blocked by the selective D_2-receptor antagonist domperidone.

The rationale for designing dual D_2-receptor and β_2-adrenoceptor agonists as drugs for the treatment of COPD

The body of evidence described above resulted in the initiation of a programme of work aimed at designing highly potent D_2-receptor agonists. The ultimate goal was to test the hypothesis that stimulation of

D_2-receptors on sensory nerve endings in the airways would inhibit afferent activity and consequently suppress reflex mediated cough, sputum production and dyspnoea in patients with COPD, thus providing a novel treatment for the symptoms of this disease.

As β_2-adrenoceptor agonists have found utility in the management of COPD, specifically in reducing breathlessness, the possibility of designing drugs which also possessed this activity was explored. The primary therapeutic effect of β_2-agonists is relaxation of airway smooth muscle, but they also have effects on inflammatory processes (30–32) and on mucociliary activity (33) that may be important in alleviating COPD symptoms. Second-generation β_2-agonists, such as salmeterol and formoterol, have a clinical duration of action in excess of 12 hours (34) and therefore the aim was to design molecules that possessed a long duration of action at β_2-adrenoceptors (and D_2-receptors). Accordingly, research efforts were focused on the identification of topically active compounds that exhibited potent agonism at both D_2-receptors and β_2-adrenoceptors and that had a long duration of action. Sibenadet (4-hydroxy-7-[2-[2-[3-(2-phenylethoxy)propylsulphonyl]ethylamino]ethyl]-1,3-benzothiazol-2(3H)-one hydrochloride) (35) was one of the first such compounds identified during this programme of research and the pre-clinical findings with this compound are described below.

RESULTS

In vitro *studies*

Detailed *in vitro* pharmacological profiling of sibenadet was performed using a range of isolated tissue systems and radioligand binding assays. These studies demonstrated that sibenadet was a potent β_2-adrenoceptor (EC_{50} of 11·2 nM in guinea-pig trachea) and D_2-receptor agonist (EC_{50} = 1·1 nM in rabbit ear artery) (36). The compound also showed good selectivity in that it was either inactive or had low activity at a range of other adrenoceptors (β_1, β_3, α_1 and α_2) and dopamine receptors (D_1, D_4, D_5) although it also had high affinity for the dopamine D_3-receptor (36). Sibenadet was also shown to have prolonged β_2 duration of action in the guinea-pig isolated, superfused trachea preparation exhibiting a recovery profile significantly longer than formoterol but shorter than salmeterol (37).

In vivo *studies*

The ability of the D_2-receptor agonist properties of sibenadet to modulate sensory nerve driven processes has been examined in a number of animal models. Sibenadet (10 $\mu g\,kg^{-1}$, i.v.) inhibited histamine-induced discharge from RARs in β-blocked dogs, an effect that was reversed by the D_2-receptor antagonist, sulpiride (38). Given this finding, it was predicted that sibenadet would block reflex-induced responses in animal model systems. Histamine aerosols when given intratracheally to lightly anaesthetized β-blocked dogs elicit a rapid rise in breathing rate, which is reflex in nature as judged by the effects of vagal cooling in this system (39).

This provided a useful model system to study breathlessness as it may be driven by activation of RARs, but also potentially via airway C-fibres, the activation of which has been shown to elicit tachypnoea in the dog (11). This histamine-induced tachypnoea was not affected by selective β_2-agonists, but was inhibited in a dose-dependent manner by sibenadet, yielding an ED_{50} of 0·8 $\mu g\,kg^{-1}$, following aerosol administration (40). The activity of sibenadet in the tachypnoea model lasted for at least 4·5 hours, which was the maximum length of time the model could be successfully run. At the end of this period, its effects were completely reversed by the peripherally acting D_2-receptor antagonist, domperidone.

Also in the anaesthetized dog, the effect of sibenadet on reflex-induced mucus secretion was investigated using a variation of the method of Phipps and Richardson (41). Ammonia vapour given to the lungs elicited large increases in mucus secretion (as judged by fucose output) from an isolated innervated loop of perfused trachea. These increases were reduced by cooling the vagi prior to ammonia challenge, an effect that could be reversed by allowing the vagi to re-warm before a subsequent ammonia challenge (42). In the β-blocked dog, sibenadet (~1 $\mu g\,kg^{-1}$ and ~10 $\mu g\,kg^{-1}$, aerosol) inhibited the ammonia-induced increases in mucus in a dose-dependent manner (42) with a similar maximal efficacy to that of vagal cooling. Sibenadet (5 $\mu g\,kg^{-1}$, aerosol) also inhibited capsaicin-induced cough in the β-blocked dog (40), a challenge chosen to mimic clinical experimental systems (43).

The effect of sibenadet on capsaicin-induced cough is of interest, as there have been recent reports of lowered cough thresholds in patients with COPD to citric acid challenge (44) as well as capsaicin (45). Sibenadet has also been shown to inhibit capsaicin-induced plasma extravasation across the trachea of the β-blocked rat (46), an effect reversed by the D_2-receptor antagonist, sulpiride. This suggests a potential inhibitory activity on C-fibre-induced effects.

Studies conducted with sibenadet in the β-blocked dog to investigate its emetic potential (the emetic effects of a number of D_2 agonists are well known) indicated that a wide therapeutic window (minimum emetic dose/ED_{50} in tachypnoea model dose = 39) exists following aerosol administration (47). There is also limited emetic potential for any swallowed component of the drug, as judged by the large dose required to induce emesis by the oral route (47). These data, together with the fact that sibenadet was optimized for topical delivery to the

lung with rapid first pass hepatic clearance, suggested that it would have a large safety margin in man.

When examined in histamine-induced bronchoconstriction models in both the dog and the guinea-pig, sibenadet administered by aerosol was shown to be a potent β_2-agonist with a long duration of action (greater than 10 hours in the dog and greater than 7 hours in the guinea-pig) (48). Comparing the ED_{50} values obtained from the β_2 curves obtained in the dog model ($0{\cdot}6\ \mu g\ kg^{-1}$) with those estimated from D_2 curves in the dog tachypnoea model ($0{\cdot}8\ \mu g\ kg^{-1}$) demonstrated that sibenadet had equivalent potency at both receptors in the same species, *in vivo*. Compounds with different ratios of β_2 to D_2 activity were also identified during the course of our research programme (49).

Effects of sibenadet on the cough reflex in man

The above pre-clinical studies provided a wealth of data on the effects of sibenadet in sensory nerve driven animal models that were designed to mimic the symptoms of COPD.

As a link between these studies and the clinical programme in patients with COPD, the effects of sibenadet on cough, induced by inhalation of nebulized distilled water, in human volunteers was investigated. This study demonstrated that sibenadet (0·45 mg via pMDI) significantly reduced cough in these volunteers (50). Given that these volunteers were β-blocked and that the selective D_2-receptor antagonist domperidone partially reversed the effects of sibenadet, it seemed reasonable to speculate that the drug was acting via stimulation of D_2-receptors on sensory nerves in the lung.

CONCLUSIONS

A range of pre-clinical studies have demonstrated that sibenadet is a potent dual D_2-receptor and β_2-adrenoceptor agonist that has a long duration of action in the dog following topical delivery to the lung. The premise that D_2-receptor activation could modulate the activity of sensory pulmonary nerves has been confirmed and the compound has been shown to be effective in a range of reflex-induced models configured to mimic the symptoms of breathlessness, excess sputum production and cough that afflict patients with COPD. These results encouraged the initiation of a clinical development programme with sibenadet for the symptomatic treatment of COPD.

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