Pharmacological Potential of PDE5 Inhibitors for the Treatment of Cystic Fibrosis

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1. Introduction

Recent basic research has aroused great interest in the therapeutic potential of phosphodiesterase type 5 (PDE5) inhibitors, such as sildenafil, vardenafil and taladafil, for the treatment of cystic fibrosis (CF). CF is the most common, life-threatening, recessively inherited disease in Caucasian populations. An estimated 1 in 2,500 Caucasian live births are affected and approximately 80,000 people in the world are diagnosed with CF. Due to mutation in the CF transmembrane conductance regulator (*CFTR*) gene [1,2], which encodes the main chloride channel expressed in epithelia, CF causes abnormal mucociliary clearance mainly in the lungs, leading to a vicious cycle of obstruction/infection/inflammation that progressively and irreversibly damages the lung tissue and architecture. Although many organs are affected in CF, pulmonary disease is the major cause of morbidity and mortality [3,4]. Despite more than two decades of intensive investigation of the genetics [1,2], pathophysiology and clinical phenotypes of CF [3,4], there is still no cure for CF. As a matter of fact, therapies have been limited to alleviating clinical manifestations. Although life expectancy and quality of life have progressively improved, CF continues to inflict major burdens and to shorten lives.

The most common disease allele, p.Phe508del (F508del), corresponding to deletion of a single phenylalanine residue at position 508 of a single polypeptide chain of 1480 amino acids, interferes with CFTR function because the mutant protein does not efficiently fold into the native protein structure. Although the mutant F508del is correctly translated, it is held back in the endoplasmic reticulum; the misfolded protein is directed towards proteosomal degradation and fails to reach the apical membrane of many epithelial cells [5]. An effective candidate drug to treat F508del-CF patients should be able to correct the localization of CFTR protein by increasing its expression at the apical membrane of epithelial cells. Indeed, it has been recognized that rescuing F508del-CFTR to the plasma membrane is followed by an improved efflux of chloride ions across the epithelium related to some residual channel activity of the mutant protein [6]. Therefore, finding a compound that promotes CFTR channel activity would be of great benefit. Searching for such compounds, we and others have demonstrated the potential of PDE5 inhibitors for the treatment of CF. Indeed, basic studies have provided evidence that PDE5 inhibitors, already

in clinical use for the treatment of erectile dysfunction and/or of pulmonary arterial hypertension, rescue F508del-CFTR trafficking [7,8] and improve its channel activity [9,10].

PDE are enzymes that regulate the intracellular levels of the second messengers, such as cyclic AMP and GMP, by controlling their rate of degradation. The enzymes catalyze the hydrolysis of the 3' cyclic phosphate bonds of adenosine (Figure 1) and/or guanosine 3'5' cyclic monophosphate.
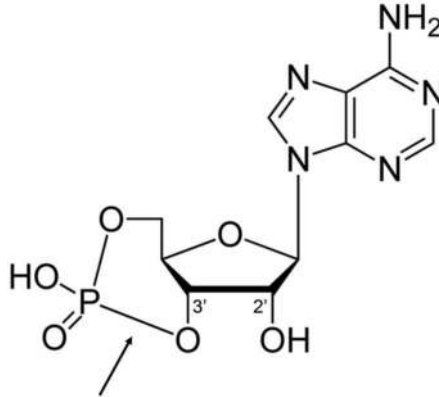

Fig. 1. **Structure of cyclic AMP.** Arrow indicates the site of hydrolyses by phosphodiesterases: the 3' cyclic phosphate bond.

Many of the early studies on cyclic nucleotides were directed toward understanding PDE activity since at that time it was much easier to measure PDE activity than either cAMP or cGMP themselves or the enzymes that catalyzed their synthesis. More recently, it became clear that there were likely to be multiple isoforms of PDEs with different kinetic and regulatory properties. They are characterized by their specificity and sensitivity to calcium-calmodulin and by their affinity for cAMP or cGMP [11]. PDEs were classified on the basis of their amino acid sequences, substrate specificities, pharmacological properties and tissue distributions.

2. Cyclic nucleotide phosphodiesterases

2.1 Isoforms of phosphodiesterases

It is now very clear that any single cell type can express several different PDE isoforms and also that the nature and localization of these PDEs are likely to be major regulators of the local concentrations of cAMP or cGMP in the cell. Eleven cyclic PDE families with varying selectivities for cAMP and/or cGMP have been identified in mammalian tissues [12-16] (Table 1).

PDEs are therefore important regulators of diverse biochemical mechanisms mediated by cAMP and /or cGMP. Despite this heterogeneity, there is a surprising degree of homology within their catalytic domains; however, slight structural differences in these domains determine whether a PDE is cAMP-specific (PDE4, PDE7, PDE8), cGMPspecific (PDE5, PDE6, PDE9) or has dual substrate specificity (PDE1, PDE2, PDE3, PDE10, PDE11) [17-18].

PDE isoenzyme	Substrate	Km (µM) cAMP	Km (µM) GMP	Tissue expression	Specific inhibitors
1	Ca^{2+}/calmodulin stimulated	80	3	Heart, brain, lung, smooth muscle, T lymphocytes, sperm	KS505a, bepril, Vinpocetine, Flunarizine and Amiodarone
2	cGMP-stimulated	30	10	Adrenal gland, heart, lung, liver, platelets	EHNA, BAY 60-7550, Oxindole and PDP
3	cGMP-inhibited cAMP-selective	0.4	0.3	Heart, lung, liver, platelets, Kidney, T lymphocytes, adipocytes, inflammatory cells	Cilostamide, Enoxamone, Milrinone, Siguazodan
4	cAMP-specific	4		Sertoli cells, kidney, brain, liver, lung, inflammatory cells	Rolipram, Roflumilast, Cilomilast, Drotaverine, ibudilast
5	cGMP-specific	150	1	Lung, platelets, vascular, smooth muscle	Sildenafil, Vardenafil, Tadalafil, Zaprinast
6	cGMP-specific		60	Photoreceptor	Dipyridamole
7	cAMP-specific, high-affinity	700	15	Skeletal muscle, heart, kidney, Brain, pancreas, T lymphocytes	BRL-50481, BC30
8	cAMP-selective	0.06		Testes, eye, liver, skeletal muscle, Heart, kidney, ovary, brain, T lymphocytes	PF-04957325
9	cGMP-specific	230	0.2	Kidney, liver, lung, brain	BAY 73-6691
10	cGMP-sensitive, cAMP-selective	0.2	13	Testes, brain	None
11	cGMP-sensitive, dual specificity	0.7	0.6	Skeletal muscle, prostate, kidney, liver, pituitary, testes and salivary glands	None

Table 1. Phosphodiesterase families and specific inhibitors

PDE1s are calcium dependent activators or regulators: they have been shown to activate cyclic nucleotide PDE in a calcium-dependent manner. PDE1s are present in many tissues and are abundant mainly in the central nervous system, heart, skeletal muscle and kidney [19-21].

PDE2 metabolizes both cGMP and cAMP although its affinity for cGMP is slightly higher than for cAMP [22]. High PDE2 activity can be found in heart [23] and brain. Lower expression of PDE2 was found in lung, placenta, liver, skeletal muscle, kidney and pancreas [24].

PDE3s are characterized by their high affinity and their ability to metabolize both cAMP and cGMP. They are also distinguished by their ability to be activated by several phosphorylation pathways including the PKA and PI3K/PKB pathways. PDE3s are moderately expressed in platelets as well as in vascular smooth muscle [25] and oocytes.

PDE4s have a higher affinity for cAMP, they are expressed in inflammatory cells such as T cells, B cells, eosinophils, neutrophils, airway epithelial cells and endothelial cells [26-28], cardiovascular tissues and smooth muscles. Differential expression of PDE4s can be modulated by inflammatory factors and expressed in lung macrophages from patients with chronic obstructive pulmonary disease (COPD).

PDE5 has a higher affinity for cGMP and was identified, isolated and characterized in rat platelets [29,30] and rat lung [31,32]. PDE5 is widely expressed in pulmonary vascular smooth muscle of pulmonary arteries and veins, bronchial blood vessels and airway smooth muscle [33]. Recent data show that PDE5 may modulate pulmonary arterial pressure induced by cardiac hypertrophy and fibrosis ([34].

PDE6s are phosphodiesterases characterized by their affinity for cGMP and are expressed in the photoreceptor outer segments of the mammalian retina, in which they mediate transduction of the light signal into an electrical response [35].

PDE7 are characterized by their high affinity and selectivity for cAMP as substrate. PDE7 protein expression is largest in T cell lines, blood T cells, epithelial cell lines, airway and vascular smooth muscle cells, lung fibroblasts and eosinophils and in neutrophils [36].

PDE8s are cAMP specific and have a very high affinity for cAMP as a substrate. PDE8s are distributed in various human tissues and are abundant in testis [37-40]. Functionally, PDE8s have been reported to be involved in regulation of T-cell activation [41], chemotaxis of activated lymphocytes [42], modulation of testosterone production in Leydig cells [43], and possibly potentiation of biphasic insulin response to glucose [44].

PDE9 is one of the more recently discovered PDE families. It is perhaps most notable as the PDE family having the highest affinity for cGMP. Further, compared with other cGMP-specific PDEs, PDE9 apparently lacks the non catalytic cGMP-binding domain, which is present in PDE5, PDE6, and also PDE2. The mRNA encoding PDE9 is well expressed in many examined human tissues, including spleen, small intestine, and brain [45,46].

PDE10 was isolated and characterized as a dual-substrate gene family in 1999 from mouse [47] as well as from human fetal lung [48] and fetal brain [49]. This PDE family was recently shown to be associated to the progressive neurodegenerative Huntington's disease (HD) since PDE10 mRNA decreases prior to the onset of motor symptoms in transgenic HD mice expressing exon 1 of the human Huntington gene [50].

PDE11 are characterized by their high affinity for both cAMP and cGMP, although kinetic characteristics for the variants are different [51-53]. PDE 11 mRNA occurs at higher levels in skeletal muscle, prostate, kidney, liver, pituitary and salivary glands, and testis.

3. PDE inhibitors as pharmacological tools in the treatment of diseases

The principle that inhibition of PDE activity could be a valid therapeutic tool is now well accepted. It is commonly accepted that concentrations of cAMP and cGMP in most cells are typically <1 to 10μM [54]. This means that a competitive inhibitor would not need to compete with very high levels of endogenous substrate in order to be effective.

The history of the PDE starts with the work of Henry Hyde Salter in 1887. It has been shown that caffeine has a bronchodilatator effect and that it was a non selective inhibitor of PDE activity. The caffeine and other xanthines have been used as therapeutic agents in respiratory diseases [55].

Inhibition of cyclic nucleotide PDEs allow cAMP/cGMP concentrations to increase within cells. Therefore, inhibition of PDE is a useful way of causing a variety of cellular effects and can influence various physiological mechanisms. Many PDE inhibitors are recognized as pharmacological agents. In fact, some compounds such as theophylline have been used as drugs in medical practice long before they were identified as PDE inhibitors. Currently, both non selective and selective PDE inhibitors are explored as therapeutic agents.

3.1 Non selective PDE inhibitors

Non selective inhibitors of the PDE such as theophylline, caffeine and papaverin have been used for more than 70 years in the western world for treatment of various diseases [56-59] and were identified as PDE inhibitors, i.e. as compounds that specifically inhibit the activity of PDE and not of other phosphohydrolases. During the last 10 years, a better understanding of physiological roles, cellular expression, specific inhibitors of the PDE isoforms, as well as of their clinical indications has been acquired. These non selective PDE inhibitors inhibit PDE competitively with low affinity and do not discriminate between PDE isozymes; both cAMP and cGMP–PDE activities are inhibited. Theophylline and other methylxantines are potent antagonists of adenosine receptors [60]. Theophylline had been prescribed for the first time in 1937 for the treatment of asthma; it is also perceived to be an orally active anti-inflammatory agent for use in asthma or COPD [57,61]. Paraxanthine, the primary metabolite of caffeine, acts through the ryanodine receptor to elevate intracellular calcium concentration and increases viability of neuronal cells in culture [62]. 3-isobutyl-1-methylxanthine (IBMX) was synthesized by Wells et al (1975), it has a much higher affinity for PDEs and at low concentrations, it preferentially inhibits cGMP-PDE over cAMP-PDE [63].

3.2 Selective PDE inhibitors

3.2.1 Inhibitors without therapeutic action

PDE2 is involved in a variety of physiological processes. The availability of PDE selective inhibitors has greatly facilitated the elucidation of PDE2 function in various tissues. One of the first specific inhibitors for PDE2 was erythro 9-(2 hydroxy-3-nonyl) adenine (EHNA) which potentiates the effects of NMDA (N-methyl-D-aspartate) activated receptors in cGMP, but has no effect on cAMP concentration [64]. EHNA is also a potent inhibitor of adenosine deaminase (ADA); it exerts a concentration dependent inhibition of the cGMP-stimulated PDE2 but does not inhibit other PDEs [65]. The strong expression of PDE2 in neurons of the hippocampus and cortex [66] suggests that this enzyme may control intraneuronal second messenger concentrations in these areas. Bayer (Germany) has developed a selective PDE2 inhibitor, the Bay 60-7550, which enhances long-term potentiation of synaptic transmission without altering basal synaptic transmission. BAY 60-7550 can improve memory functions by enhancing neural plasticity [67,68].

3.2.2 Inhibitors with therapeutic action

Some selective PDE inhibitors act directly on the catalytic site of PDE1s, such as vinpocetine. This PDE inhibitor has been used in memory loss [69] and in treating detrusor instabilities and urgency incontinence [70]. PDE inhibitor can improve neural plasticity or restore this function in different neurological conditions [71,72]. Vinpocetine treatment was also shown to revert the effects of early alcohol exposure in learning performance in the water maze [73]. It was recently demonstrated that vinpocetine has a strong anti-inflammatory effect [74]. This new action of vinpocetine, combined with its potential to enhance neuronal plasticity suggest that this drug may have beneficial effects in conditions such as Alzheimer and Parkinson diseases where inflammation and poor neuronal plasticity are present [75].

There are a relatively large number of PDE3 selective inhibitors including milrinone, cilostamide and cilostazol, which were identified as potential therapeutic tools in cardiovascular disease and asthma. Inhibition of PDE3 activity increase L-type Ca^{2+} currents in cardiomyocytes isolated from human, rat and frog heart, an effect that contributes to the positive inotropic effects of these inhibitors [76]. Milrinone has an inotropic and vasodilator effect for "wet and cold" heart failure [77], a case of heart failure with congestion and hypoperfusion [78]. It has been reported that the combination of inhaled and intravenous milrinone could be an effective treatment of secondary pulmonary hypertension in high-risk cardiac valve surgery patients [79].

PDE4 inhibitors have been developed for the treatment of asthma and COPD, diseases characterised by inflammatory and immune responses [80]. Rolipram is a highly selective first generation PDE4 inhibitor that has been used for many years as a research tool to investigate the role of PDE4. Several studies have shown that rolipram inhibits neutrophilic and eosinophilic inflammation [81]; it proved to be an effective antidepressant, but side effects such as nausea and gastro-intestinal disturbance terminated its clinical development [82]. Roflumilast was beneficial, as assessed by improvement in lung function, even when added to a long acting β_2 agonist or a long acting inhaled antimuscarinic [83].

The use of inhibitors of PDE5 (sildenafil (Viagra; Pfizer Inc, US), vardenafil (Levitra; GlaxoSmithKline, UK) and tadalafil (Cialis; Eli Lilly, US)) in the treatment of male erectile dysfunction is the first commercial success for PDE inhibitors. Sildenafil (under the tradename Revatio) and tadalafil (under the tradename Adcirca) have also been approved for the treatment of pulmonary arterial hypertension (PAH). PDE5 is a cGMP-specific phosphodiesterase encoded by a single gene. Recent data show that PDE5 may modulate pressure-induced cardiac hypertrophy and fibrosis [34]. Although sildenafil has an acceptable degree of selectivity, increased specificity for PDE5, particularly over PDE1 and PDE6 will reduce or eliminate the incidence of visual disturbances associated with the flushing and headaches that are observed with sildenafil [84]. In the case of all the other PDE5 inhibitors that have been described in the peer-reviewed literature, improvements in selectivity were determined empirically, and compounds were optimized on the basis of structure- activity explorations of the chemical series in question. PDE5 is abundantly expressed in lung tissue and appears to be up regulated in PAH [85,86]. PDE5 is involved in endothelial dysfunction by inactivating cGMP, the second messenger of the nitric oxide (NO) pathway in the pulmonary vasculature [85-87]. It has been reported that sildenafil and vardenafil raise hippocampal cGMP levels and improve memory in aged rats [88] and mice [89].

The PDE7 family is composed of two genes coding for high-affinity, rolipram-insensitive, cAMP-specific enzymes. The presence of high concentrations of PDE7 mRNA in the human striatum and dentate gyrus suggests that selective inhibitors could be used to increase cAMP concentration in these areas without some of the side effects associated with PDE4 inhibition [40,90,91]. Several distinct PDE7 inhibitors have been reported [92,93]; however, their effects on central nervous system (CNS) function have yet to be described. It has been shown that selective inhibition of PDE7 or dual PDE4/7 inhibition may provide a novel therapeutic approach for the treatment of chronic lymphocytic leukemia (CLL) by enhancing killing and increasing specificity for CLL cells [94].

The company Pfizer reported on a small molecule called PF-04957325 that selectively inhibits PDE8 with an *in vitro* IC50 of 0.7nM against PDE8A, of 0.2nM against PDE8B, and >1.5µM against all other PDE isoforms [95]. PDE8-selective inhibitors might be used to correct adrenal insufficiency, and a PDE8 activator might be used to treat Cushing's syndrome [96].

4. Pharmacological potential of PDE inhibitors for the treatment of cystic fibrosis

As an important second messenger signaling molecule, cAMP controls a wide variety of eukaryotic and prokaryotic responses to extracellular cues [97]. For cAMP-dependent signaling pathways to be effective, the intracellular cAMP concentration is tightly controlled at the level of both of synthesis and degradation. CF is characterized by defective cAMP-dependent chloride conductance in epithelial cells and is caused by a defect in the targeting of the chloride channel CFTR.

4.1 Non selective PDE inhibitors

Non specific inhibitors of the PDE such as IBMX, theophylline and DPMX (7-methyl-1,3 dipropyl xanthine) have been shown to activate normal and mutated CFTR chloride channels in epithelia [98]. It is well known that the methylxanthines, found naturally in tea, coffee and cocoa, stimulate the central nervous system, relax bronchial smooth muscle, and stimulate cardiac muscle. These purine derivatives function as adenosine receptor antagonists and as PDE inhibitors. Due to impact on the cAMP pathway and activity at low concentrations, studies have been done looking at their effect on the cAMP activated CFTR channel. The PDE inhibitor, IBMX also functions as an adenosine receptor antagonist. It has been reported that IBMX increases the CFTR chloride current in Xenopus oocytes expressing the F508del-CFTR [99]. In 1993, when studying CF nasal bronchial epithelial tissues with F508del-CFTR, Grubb et al. found that IBMX (5 mM) associated to forskolin (0.01 mM) did not stimulate chloride efflux *in vitro* [100]. Haws et al. studied the effect of IBMX and 8-cyclopentyl-1,3-dipropylxanthine (CPX), another non specific PDE and an A1 adenosine receptor antagonist, on stably transfected cells with F508del-CFTR [101]. In this study, both IBMX (5 mM) and CPX potentiated the effect of forskolin on CFTR-mediated efflux of ^{125}I by 2.5-fold. There was a 7-fold increase in cAMP levels associated with IBMX treatment, but not CPX treatment. A potentiation by IBMX of prostaglandin E (PGE2)-induced HCO_3^- secretion has been reported in the rat duodenum *in vivo* [102,103].

4.2 Selective PDE inhibitors

PDE inhibitors increase cAMP by inhibiting one or more enzymes involved in cAMP degradation. Cyclic AMP-activated PKA mediates phosphorylation of CFTR and increases the open probability of the CFTR channel. Drugs in this class include amrinone and milrinone. These drugs also cause vasodilation, which may be beneficial for the CF airways. In 1991, Drumm et al. showed that inhibiting PDE had a larger effect on CFTR activation than have adenylate cyclase stimulants [99]. Using airway epithelial cell lines expressing wild-type CFTR, Calu-3 and 16HBE cells, it has been found that, at 100μM concentrations, PDE 3 inhibitors (milrinone, amrinone) without adenylate cyclase activators, stimulate   chloride efflux 13.7-fold [104]. They found no effect on chloride efflux by IBMX, a non specific PDE, by rolipram, a PDE4 inhibitor or by dipyridamole, a PDE5 inhibitor. The increase of channel efflux by the type 3 PDE inhibitor was not associated with a significant rise in cAMP concentrations but it was inhibited by protein kinase A inhibitors (H-8 and Rp-cAMPS), suggesting that it might work through a more distal signal. Kelley et al. also looked at endogenous CFTR in transformed nasal polyp tissue of patients homozygous for F508del (CF-T43) [105]. They found that, when administered in the presence of a β-agonist (isoproterenol) and protein kinase A activator, milrinone and amrinone, at 100μM concentrations, increased chloride efflux by 19-61% from baseline. Mice homozygous for F508del Cftr were administered with a combination of milrinone (100 μM) and forskolin (10 μM) [106]. This combination of drugs resulted in an increased magnitude of the murine nasal potential difference (PD). The implications of this study are exciting; but the effect has not been confirmed by others [107].

It has been shown that CFTR has a major role in the regulation of duodenal HCO_3^- secretion [108]. Furthermore, O'Grady et al. [109] showed that both PDE1 and PDE3 are involved in the activation of CFTR in T84 cells and human colonic epithelial cells. In 2007, Hayashi M et al. [110] suggested that PDE1 and PDE3 are involved in the regulation of duodenal HCO_3^- secretion and that the response to PGE2 is associated with both PDE1 and PDE3, while the response to NO is mainly modulated by PDE1 [110]. McPherson et al. showed that a selective cyclic nucleotide PDE5 inhibitor partially corrected defective L-adrenergic stimulation of mucin secretion in CFTR antibody-inhibited submandibular cells. The PDE5 inhibitor did not increase cAMP levels, nor did it potentiate isoproterenol-induced cAMP rise [111]. Of note, Dormer et al. (2005) demonstrated that the PDE5 inhibitor sildenafil (Viagra) also acts as a pharmacological chaperone. Because sildenafil is approved for clinical use, they speculated that their data might speed up the development of new therapies for CF [7].

5. The clinical pharmacokinetics of PDE5 inhibitors

Lung tissue is a rich source of PDE, including PDE5, the major function of which is acceleration of the decay of cGMP [112].

5.1 Sildenafil

Sildenafil citrate was the first selective PDE5 inhibitor approved for the treatment of erectile dysfunction. Sildenafil, however, is only approximately 10-fold as potent for PDE5 as for PDE6, which is found in the photoreceptors of the human retina. This lower selectivity toward PDE6 is presumed to be the cause for color vision abnormalities observed with high doses or plasma levels of sildenafil.

Sildenafil is relatively lipophilic with a weakly basic center in the piperazine tertiary amine, resulting in only partial ionization at physiological pH. Following oral administration, sildenafil is rapidly absorbed, reaching peak plasma concentrations within 1 hour (range, 0.5-2 hours). The first-order absorption rate constant was estimated as 2.6 hours^{-1} based on population pharmacokinetic data in patients with erectile dysfunction [113]. Administration of sildenafil after a high-fat meal caused reductions in the rate of absorption and extent of systemic exposure. The time-to-peak (t_{max}) was delayed by approximately 1 hour, and maximum concentration (C_{max}) was reduced by 29%. The systemic exposure of sildenafil after a high-fat meal was reduced by 11% [114].

Sildenafil is highly bound to plasma proteins, and the protein binding is independent of drug concentrations. After intravenous administration, the mean steady-state volume of distribution of sildenafil is 105 L, which substantially exceeds the total volume of body water (approximately 42 L), indicating distribution into tissues and possibly binding to extravascular proteins. Sildenafil is extensively metabolized, without unchanged sildenafil being detected in either urine or feces. After an oral dose, metabolites are predominantly excreted into the feces (73%-88%) and to a lesser extent into the urine (6%-15%) [115]. Plasma concentrations of sildenafil was reported to decline biexponentially, with a mean terminal half-life of 3 to 5 hours, independent of the route of administration [114]. Sildenafil is primarily metabolized by the cytochrome P-450 (CYP) isoenzyme CYP3A4 and to a lesser extent CYP2C9 [116]. Sildenafil is extensively metabolized, with more than 12 metabolites identified.

The principal routes of metabolism are N-demethylation, oxidation, and aliphatic hydroxylation [115]. Plasma concentrations of N-demethylation are approximately 40% that of sildenafil, so that the metabolite accounts for approximately 20% of the pharmacological effects of sildenafil. The metabolite profile is qualitatively similar after intravenous and oral administration, but higher concentrations of N-desmethyl sildenafil after oral administration indicate the important role of first-pass metabolism in the metabolite formation.

5.2 Vardenafil

Vardenafil hydrochloride was the first second generation PDE5 inhibitor approved for the treatment of erectile dysfunction. Vardenafil has a high selectivity for the inhibition of PDE5 compared with the other known phosphodiesterases [117,118]. Unlike sildenafil and tadalafil, vardenafil was developed from the outset specifically to treat erectile dysfunction.

Vardenafil is rapidly absorbed, with plasma concentrations being detected in all subjects within 8 to 15 minutes after oral administration.

Peak plasma concentrations were observed 0.25 to 3 hours after administration, with a median of 0.7 hours for the 20 and 40 mg dose level, and slightly later, with 0.9 hours for the 10 mg dose level [117,119]. The absolute bioavailability of vardenafil was described as approximately 15%. Vardenafil pharmacokinetics is largely unaffected by food containing moderate amounts of fat. Minimal changes (<15%) in mean vardenafil C_{max} and no change in median t_{max} were observed when vardenafil was administered with a moderate-fat evening meal compared to dosing on an empty stomach. When 20mg oral vardenafil was administered immediately after consumption of a high-fat breakfast, the mean C_{max} was 18% lower and the median t_{max} was delayed by 1 hour.

Based on *in vitro* investigations in human plasma, approximately 93% to 95% of the drug is bound to plasma proteins, approximately 80% to albumin, and 11% to α1-acid glycoprotein [120]. It was also demonstrated that the binding to plasma proteins was fully reversible in all the tested species and was concentration independent. The major metabolite of vardenafil has similar protein-binding properties as the parent drug, with a bound fraction of 93% to 95%. The volume of distribution estimate for vardenafil after intravenous administration is relatively high, 208 L, implying extensive drug distribution into tissues.

Vardenafil is extensively metabolized, with more than 14 metabolites identified. The major metabolite, M1, and 2 minor metabolites, M4 and M5, as well as their respective glucuronides, are all a result of the degradation of vardenafil's piperazine ring. M1 is N-desethyl vardenafil, M4 is reduced by a 2-carbon fragment of the piperazine ring of vardenafil, and M5 is the N-desethyl derivative of M4. Metabolism is predominantly mediated by CYP3A4 and to a smaller extent by CYP3A5 and CYP2C isoforms. All 3 metabolites have pharmacologic activity. The major circulating metabolite, M1, has 28% of vardenafil's potency for PDE5 inhibition, while M4 and M5 possess 5.6% and 4.9%, respectively [120].

5.3 Tadalafil

Tadalafil is a selective and potent inhibitor of PDE5 with an IC50 of 0.94 nM. It exhibits high selectivity toward PDE5 compared to other PDEs. Tadalafil is structurally different from both sildenafil and vardenafil, and the different structures are reflected in distinct differences in the clinical pharmacology profiles of these drugs [121]. Like sildenafil, tadalafil was developed initially for use in cardiovascular disease and was subsequently used for the treatment of erectile dysfunction [122]. Tadalafil was the last of the 3 PDE5 inhibitors approved for erectile dysfunction.

Tadalafil is rapidly absorbed after oral administration with a median time to reach peak plasma concentration of 2 hours (range, 0.5-6 hours) [118,121]. Absolute bioavailability of tadalafil following oral dosing has not been reported, but at least 36% of the dose is absorbed from an oral solution. The time course of oral absorption could successfully be modeled by a rapid first-order process. Population estimates of the first-order absorption rate constant from phase II and phase III studies are 1.75 and 1.86 hours^{-1}, respectively [123]. The absorption and pharmacodynamic properties of tadalafil are not affected by either food or alcohol, and thus the drug can be administered without regard for food or alcohol consumption [124]. Smoking and body mass index had a weak effect on the pharmacokinetics of tadalafil. It has been reported that the clinical response to tadalafil may be evident as early as 16 minutes and may persist for up to 24 to 36 hours post dose [124,125]

Tadalafil has an apparent volume of distribution of 60 to 70 L, with an interindividual variability of 40% to 50%. This indicates that tadalafil is distributed into tissues. Plasma protein binding was reported as 94%, with α1-acid glycoprotein and albumin as principal binding proteins. A population pharmacokinetic analysis in patients taking tadalafil suggests a body weight dependency of the volume of distribution at steady state.

Tadalafil is excreted primarily as inactive metabolites, mainly in the feces and to a lesser extent in urine. The mean elimination half-life for tadalafil was 17.5 hours, and the mean

apparent oral clearance was 2.5 L/h in healthy subjects [126]. The nearly exclusive elimination via hepatic metabolism and the relatively low value for oral clearance indicate that tadalafil has a low intrinsic clearance with regard to hepatic metabolism and can be classified as a drug with low hepatic extraction ratio.

Tadalafil is primarily metabolized by CYP3A4 to a catechol metabolite, which further undergoes extensive methylation and glucuronidation to form methylcatechol and methylcatechol glucuronide metabolites. This was confirmed by interaction studies with rifampin as potent CYP3A inducer and

ketoconazole as a potent CYP3A inhibitor. The main circulating metabolite in plasma is methylcatechol glucuronide, which has a ≥ 10 000-fold less affinity for PDE5 than the analogue drug, tadalafil, and is thus expected to be clinically inactive at observed metabolite concentrations [126]. Several other inactive metabolites have also been identified in plasma, urine, or feces.

5.4 Comparison of PDE5 inhibitors

Although the 3 currently available PDE5 inhibitors, sildenafil, vardenafil, and tadalafil, have all shown to be effective in the treatment of erectile dysfunction, there are distinct differences between the compounds regarding their selectivity and specificity for PDE inhibition with consequences especially for the safety profile but also biopharmaceutic and pharmacokinetic disparities that largely affect the efficacy profile of these compounds. Sildenafil and vardenafil are very similar in terms of their chemical structure, whereas tadalafil with a methyldione structure differs markedly from sildenafil and vardenafil (Figure 2). These chemical similarities and differences are also reflected in similarities and dissimilarities of their clinical pharmacokinetics.

All 3 PDE5 inhibitors are rapidly absorbed after oral administration, with peak concentrations reached slightly earlier for vardenafil compared to sildenafil and tadalafil. Although no clear concentration-effect relationships have been established for any of the 3 PDE5 inhibitors, rapid absorption is considered an essential for a rapid onset of efficacy. Administration of a high-fat meal had no significant effect on the rate and extent of absorption of tadalafil but decreased the rate of absorption for sildenafil and vardenafil. All 3 drugs are lipophilic and have a volume of distribution larger than the volume of total body water, indicating tissue uptake and binding. Furthermore, all 3 compounds are highly protein bound, with free plasma concentration fractions of only 4% to 6%.

The major route of elimination for all PDE5 inhibitors is hepatic metabolism, with renal excretion of unchanged drug accounting for 1% or less of the elimination pathways. Based on their relatively high systemic clearance after intravenous administration, sildenafil and vardenafil can be classified as non restrictively cleared drugs with intermediate to high hepatic extraction ratio. The relatively comparable distribution volumes together with the substantial differences in systemic clearance among the PDE5 inhibitors result in distinct differences of the elimination half-life, 3 to 5 hours for sildenafil and vardenafil compared to 17.5 hours for tadalafil. Tadalafil, however, has been detected in plasma even 5 days after oral administration due to its long half-life. This suggests the possibility of accumulation if taken regularly and in short intervals, which may result in an increased risk of side effects with the excessive use of this PDE5 inhibitor.
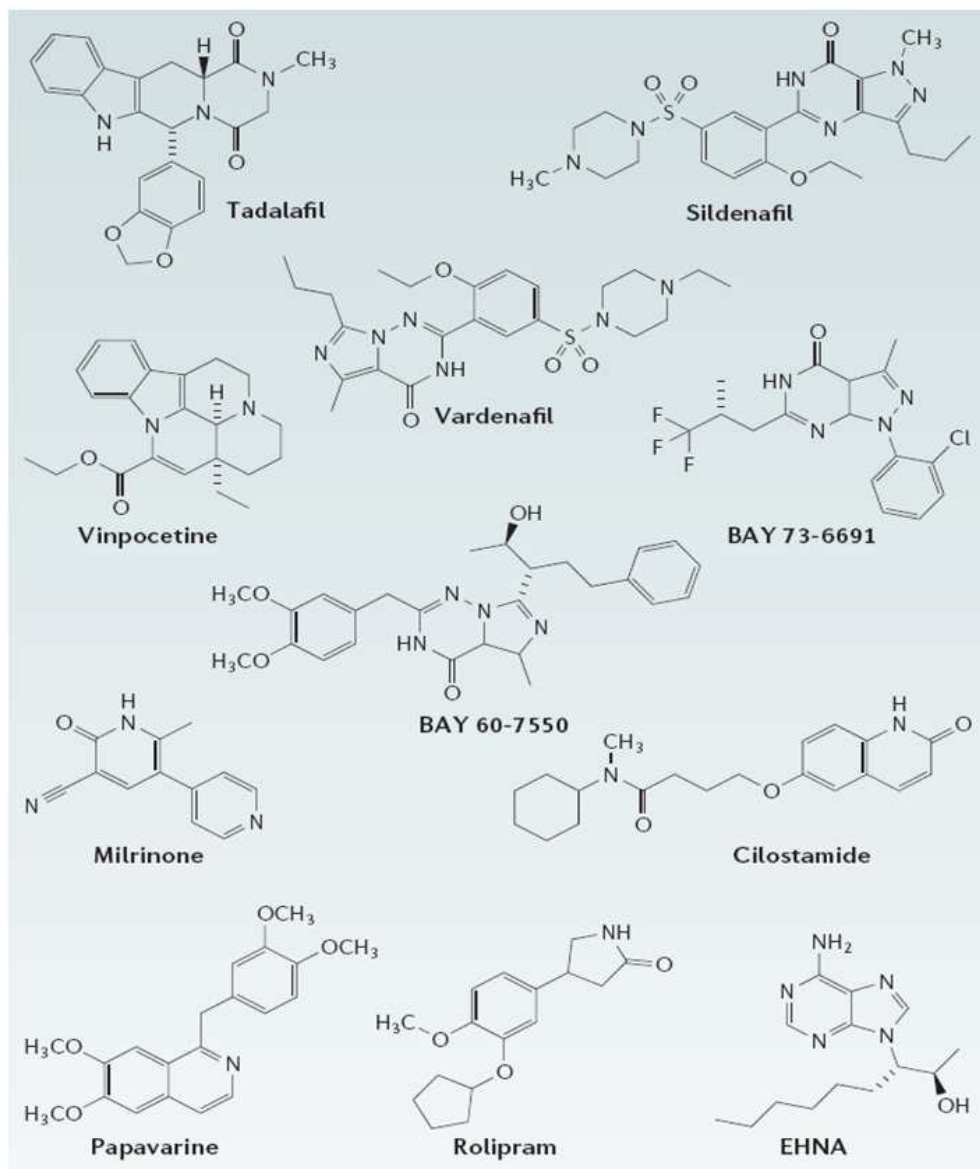
Fig. 2. **Structures of selected examples of phosphodiesterase inhibitors.** The figure shows various selective phosphodiesterase (PDE) inhibitors mentioned in this chapter. Of these, the PDE5 inhibitors sildenafil, vardenafil and tadalafil have been approved for treatment of erectile dysfunction. Sildenafil and vardenafil have also recently been approved as a treatment for pulmonary hypertension.

6. Administration of PDE5 inhibitors at clinical doses activates defective chloride transport in CF

At present, many efforts are focused on CFTR pharmacotherapy which corrects the abnormal protein pharmacologically by various approaches such as the direct correction of stop codon mutations, CFTR channel activation, or correction of CFTR trafficking defects.

High-throughput screening (HTS) has been used to identify molecules that increase F508del-CFTR activity [127,129]. Such molecules have been categorized according to whether they alleviate the folding/cellular processing defect (correctors) or increase the responsiveness of F508del-CFTR channels already present in the membrane to cAMP activation (potentiators). Sildenafil has also been shown to correct F508del-CFTR processing when used at high micromolar concentrations [7].

To test the hypothesis that PDE5 inhibitors (sildenafil, vardenafil and taladafil) are able to restore transepithelial ion transport abnormalities of the F508del-CFTR protein, we have conducted experimental studies [9,10] in CF mice homozygous for the F508del mutation [130] and in their corresponding wild-type homozygous normal mice. The F508del-Cftr mouse model has been chosen because F508del is the most common and one of the most severe CF mutation and because the mouse model recapitulates, at different levels, the human disease. Epithelia of the F508del-CF mouse model are characterized by defective electrolyte transport, and *Pseudomonas aeruginosa* lipopolysaccharide (LPS) exposure mimics several aspects of CF airway epithelial inflammation such as increased pro-inflammatory cytokines, most notably interleukin (IL)-8, IL-6, and Tumor Necrosis Factor (TNF)-α, and neutrophil infiltrate cells.

In our protocols, CFTR function has been assessed *in vivo* by measuring the transepithelial nasal PD, a delicate technique that has been increasingly used as an index of therapeutic efficacy in novel fundamental therapies, either in animal models [9,10,131] or in CF patients [132]. Our results provide clear evidence that intraperitoneal injection of PDE5 inhibitors (Figure 3), at clinical doses, to F508del-CF mice interact with CFTR, propping open the mutant protein to allow a normal flow of chloride ions across the epithelium of nasal mucosa, thereby completely restoring the decreased or even abolished CFTR-dependent chloride transport [9]. In F508del mice, but not in *Cftr* knockout mice, the chloride conductance, evaluated by perfusing the nasal mucosa with a chloride-free solution in the presence of amiloride and with forskolin, is corrected 1 h after sildenafil administration. A more prolonged effect, persisting for at least 24 h, is observed with vardenafil. Moreover, vardenafil, but not sildenafil, is able to stimulate chloride transport associated with normal wild-type Cftr protein [9]. The forskolin response is increased after treatment with sildenafil or vardenafil in wild-type and in F508del mutant animals. In F508del mice, the chloride conductance in the presence of 200 μM DIDS (4-4'-diisothiocyanostilbene-2,2'-disulphonic acid), an inhibitor of alternative chloride channels, was much higher after sildenafil injection than following placebo treatment (Figure 4). No effect on the sodium conductance was detected in any group of animals. Altogether, these data provide preclinical evidence that sildenafil and vardenafil stimulate, by a direct and not a by-pass effect, chloride transport activity of F508del-CFTR protein.
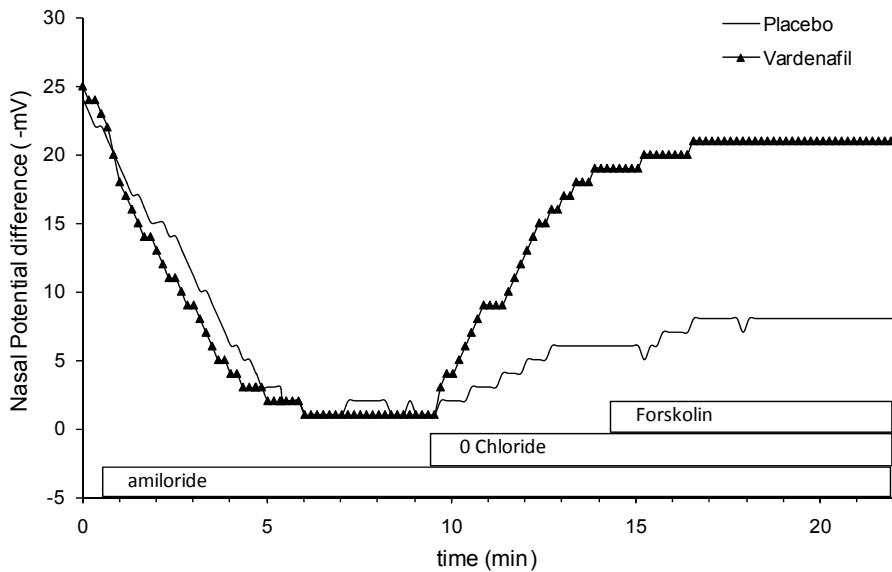
Fig. 3. Influence of vardenafil (24h after a single therapeutic dose) on ion transport evaluated by the nasal potential difference (PD) in F508del-CF mice. Chloride conductance in response to perfusion of the nasal mucosa with a solution without chloride and to forskolin is dramatically increased as compared to placebo-treated CF mice.
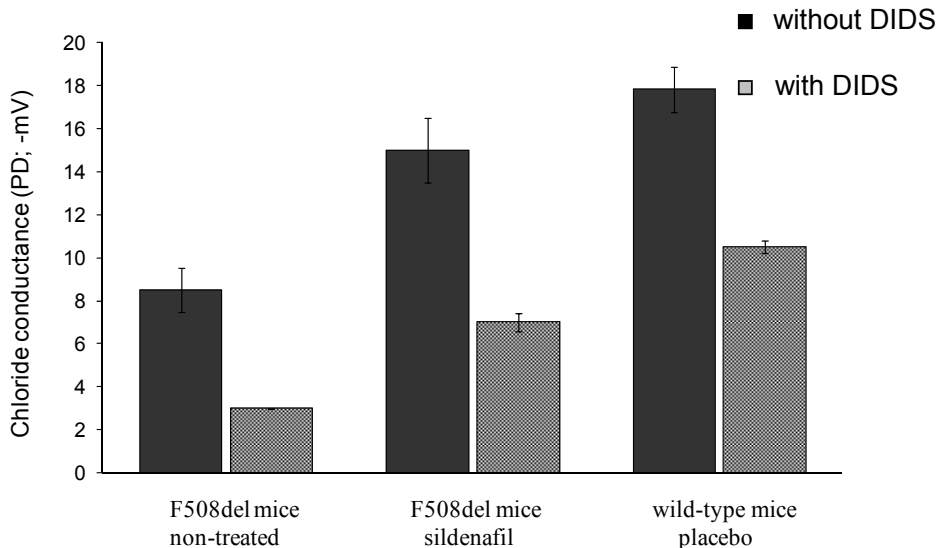

Fig. 4. Influence of sildenafil on Cftr -dependent chloride conductance evaluated by the nasal potential difference (PD) in the presence or the absence of DIDS, an inhibitor of alternative chloride conductance. Increased DIDS-insensitive conductance after sildenafil treatment reflects activation of Cftr function.

More recently, using a nebulizer setup specifically developed for mice (Figure 5), we have demonstrated that administration of PDE5 inhibitors through a single inhalation exposure is able to locally activate Cftr protein and correct the basic defects in CF [10] and that the effect lasts for at least 8 h (Figure 6). Our data have identified the inhalational route as a potential therapy for PDE5 inhibitors in CF. Consistent with our results, it has recently been demonstrated that the inhalation route of administration for vardenafil is associated with an acceptable safety profile. Apart from brief coughing on inspiration, no clinically significant changes in blood pressure or heart rate and no serious adverse events were recorded [133]. Inhalation drug therapy has several potential advantages over oral and intravenous routes, including rapid onset of pharmacological action, minimized systemic adverse effects and reduced effective drug doses compared to the same drug delivered orally [134]; this greatly highlights the impact of our work for translational science.
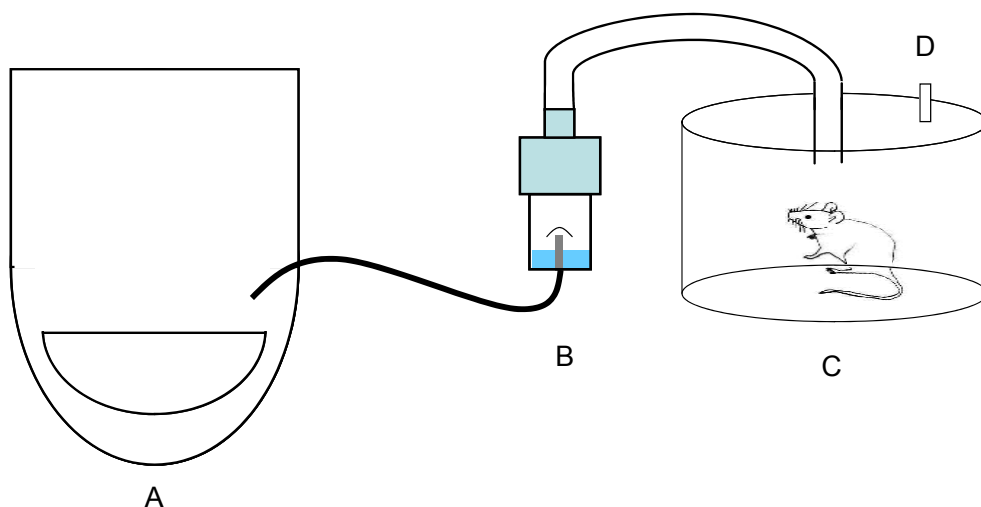
Fig. 5. Schematic representation of the whole-body immersion inhalation chamber setup we developed for a single mouse. (A) compressor, (B) nebulizer, (C) inhalation chamber with (D) expiratory gate.
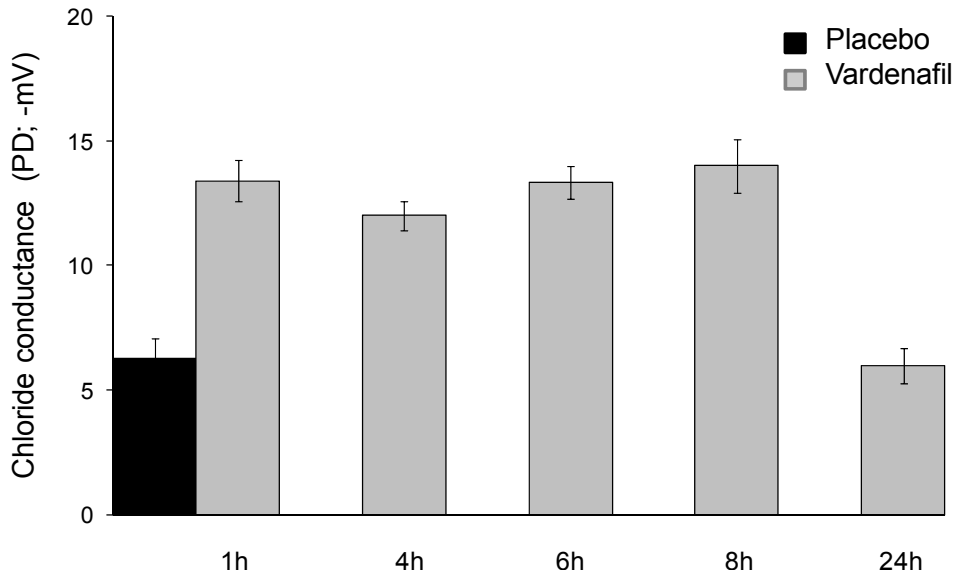
Fig. 6. Duration of the correcting effect of inhaled vardenafil on chloride conductance, evaluated by nasal potential difference (PD) in F508del-CF mice 1, 4, 6, 8 and 24h after a single nebulisation with placebo or with vardenafil. The correcting effect of vardenafil lasts at least 8 h after inhalation.

7. Intraperitoneal administration of PDE5 inhibitors administration at clinical doses attenuates exaggerated inflammatory responses in CF in vivo conditions

Another important goal of mutation-specific CF treatment is attenuation of exaggerated lung inflammatory responses [134-137]. As lung inflammation plays a major role in morbi-mortality in CF, identifying a therapeutic strategy that combines ability to correct the basic ion transport defect and to reduce dysregulated inflammatory responses is very exciting and promising. It has been reported that sildenafil reduces neutrophil lung infiltration in murine airways infected with *P. aeruginosa* [138]. In addition, toxicological studies have shown that sildenafil pretreatment attenuates acrolein-triggered airway inflammation associated with mucin overproduction [139].

More recently, we have found that vardenafil, selected as a representative PDE5 inhibitor for its longer-lasting Cftr activating effect, modulates the vicious circle of lung inflammation and attenuates the expression of pro-inflammatory cytokines and chemokines and cell infiltrates in the bronchoalveolar lavage (BAL) of CF and wild-type mice [140] Our data indicate that intraperitoneal administration of a single pharmacological dose (0.14 mg/kg body weight) of vardenafil is followed by a reducing response in cell infiltrate and in the biosynthesis of several biomarkers of the inflammatory response. Most notably, levels of CCL-2 (chemokine C-C motif ligand), a cytokine playing a key role in the contribution of macrophages in the inflammatory response [136], are significantly reduced in the BAL fluid after vardenafil treatment, particularly in CF animals (Figure 7).
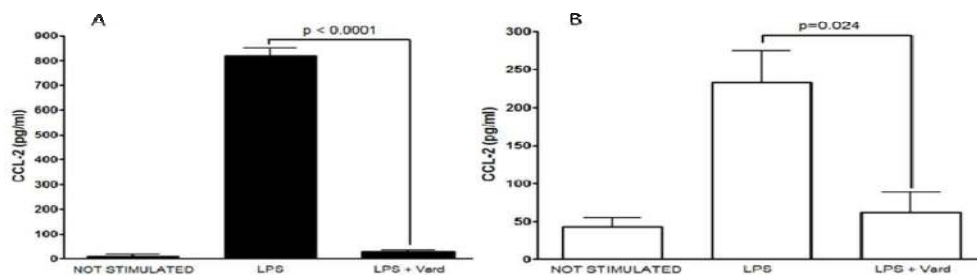
Fig. 7. Anti-inflammatory effect of *in vivo* treatment, by intraperitoneal injection, of a single therapeutic dose of vardenafil (vard) to F508del-CF (A) and wild-type (B) mice on the lipopolyssaccharide (LPS of *P. aeruginosa*) induced inflammatory response. Biosynthesis of CCL-2 is significantly reduced in the bronchoalveolar lavage (BAL) of vardenafil-treated CF and non-CF animals.

The mechanism of action of vardenafil as an anti-inflammatory agent in CF as well as the target-effector cells involved in these responses are under investigation by our group. Altogether, our data indicate that PDE5 inhibitors have a strong therapeutic potential for treating CF. A clinical trial aimed at investigating the safety and efficacy of sildenafil in CF lung disease is listed on www.clinicaltrials.gov (NCT00659529).

8. Conclusions

There is still no cure for CF. The CF patient may benefit from today's privileged strategy which consists on targeting a pharmacological mutation-specific treatment. Currently candidate molecules suitable for CFTR pharmacotherapy are either being sought after or under investigation. Based on the high prevalence of *F508del-CFTR* mutation – more than two-thirds of patients with CF carry at least one copy of the allele -, strategies to rescue the functional status of the mutated protein will benefit most of the CF population. As PDE5 inhibitors such as sildenafil, vardenafil and tadalafil are able to correct transepithelial ion transport abnormalities and to limit exaggerated inflammatory responses related to the presence of F508del-CF protein, the drugs are promising compounds for fundamental pharmacotherapy in CF. Since the drugs are in clinical use, therapeutic approaches to address F508del-CFTR defects by PDE5 inhibitors could be considered as a 'low-hanging fruit' strategy in the drug discovery tree. The fact that such compounds have been approved for other therapeutic indications could speed up their development as CF therapeutics, as compared to other agents that are under investigation only for CF therapy and for which further exploratory studies are needed before being streamed towards clinical testing.

In summary, CFTR correction with PDE5 inhibitors is a promising therapeutic approach based on functional correction of F508del-CFTR activity and on a possible anti-inflammatory action in F508del mice. The effects of these compounds on other CF mutation classes remain to be assessed.

9. Acknowledements

10. References

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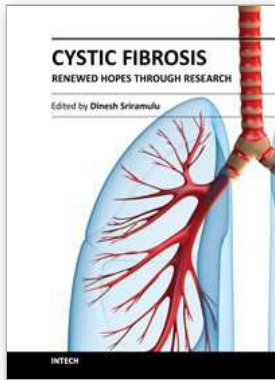
Cystic Fibrosis - Renewed Hopes Through Research

Edited by Dr. Dinesh Sriramulu

Living healthy is all one wants, but the genetics behind creation of every human is different. As a curse or human agony, some are born with congenital defects in their menu of the genome. Just one has to live with that! The complexity of cystic fibrosis condition, which is rather a slow-killer, affects various organ systems of the human body complicating further with secondary infections. That's what makes the disease so puzzling for which scientists around the world are trying to understand better and to find a cure. Though they narrowed down to a single target gene, the tentacles of the disease reach many unknown corners of the human body. Decades of scientific research in the field of chronic illnesses like this one surely increased the level of life expectancy. This book is the compilation of interesting chapters contributed by eminent interdisciplinary scientists around the world trying to make the life of cystic fibrosis patients better.

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