

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Pharmacological Potential of PDE5 Inhibitors for the Treatment of Cystic Fibrosis

Bob Lubamba, Barbara Dhooghe, Sabrina Noël and Teresinha Leal
*Louvain Centre for Toxicology and Applied Pharmacology,
Université Catholique de Louvain, Brussels,
Belgium*

1. Introduction

Recent basic research has aroused great interest in the therapeutic potential of phosphodiesterase type 5 (PDE5) inhibitors, such as sildenafil, vardenafil and tadalafil, 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.

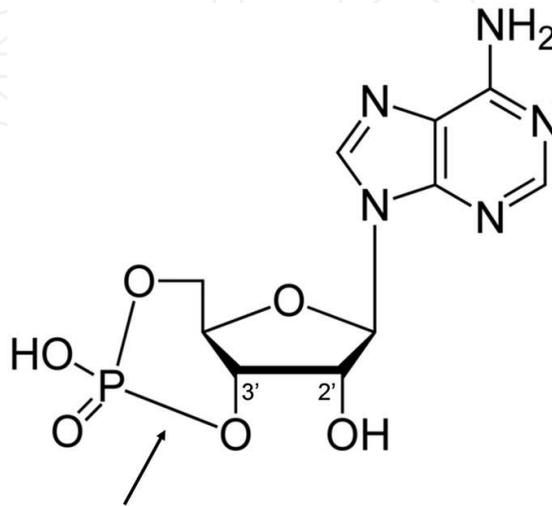


Fig. 1. **Structure of cyclic AMP.** Arrow indicates the site of hydrolyses by phosphodiesterases: the 3' cyclic phosphate bond.

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), cGMP-specific (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 Rolipram, Roflumilast, Cilomilast, Drotaverine, ibudilast Sildenafil, Vardenafil, Tadalafil, Zaprinast
4	cAMP-specific	4		Sertoli cells, kidney, brain, liver, lung, inflammatory cells	Dipyridamole
5	cGMP-specific	150	1	Lung, platelets, vascular, smooth muscle	BRL-50481, BC30
6	cGMP-specific		60	Photoreceptor	PF-04957325
7	cAMP-specific, high-affinity	700	15	Skeletal muscle, heart, kidney, Brain, pancreas, T lymphocytes	BAY 73-6691
8	cAMP-selective	0.06		Testes, eye, liver, skeletal muscle, Heart, kidney, ovary, brain, T lymphocytes	None
9	cGMP-specific	230	0.2	Kidney, liver, lung, brain	None
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 10 μ M [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 bronchodilator 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 methylxanthines 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 ¹²⁵I 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 HCO₃⁻ 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 100 μ M 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 100 μ M concentrations, increased chloride efflux by 19-61% from baseline. Mice homozygous for F508del Cfr were administered with a combination of milrinone (100 μ M) and forskolin (10 μ M) [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 HCO₃⁻ 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 HCO₃⁻ 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 α 1-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 α 1-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 $\geq 10\ 000$ -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 methylidione 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.

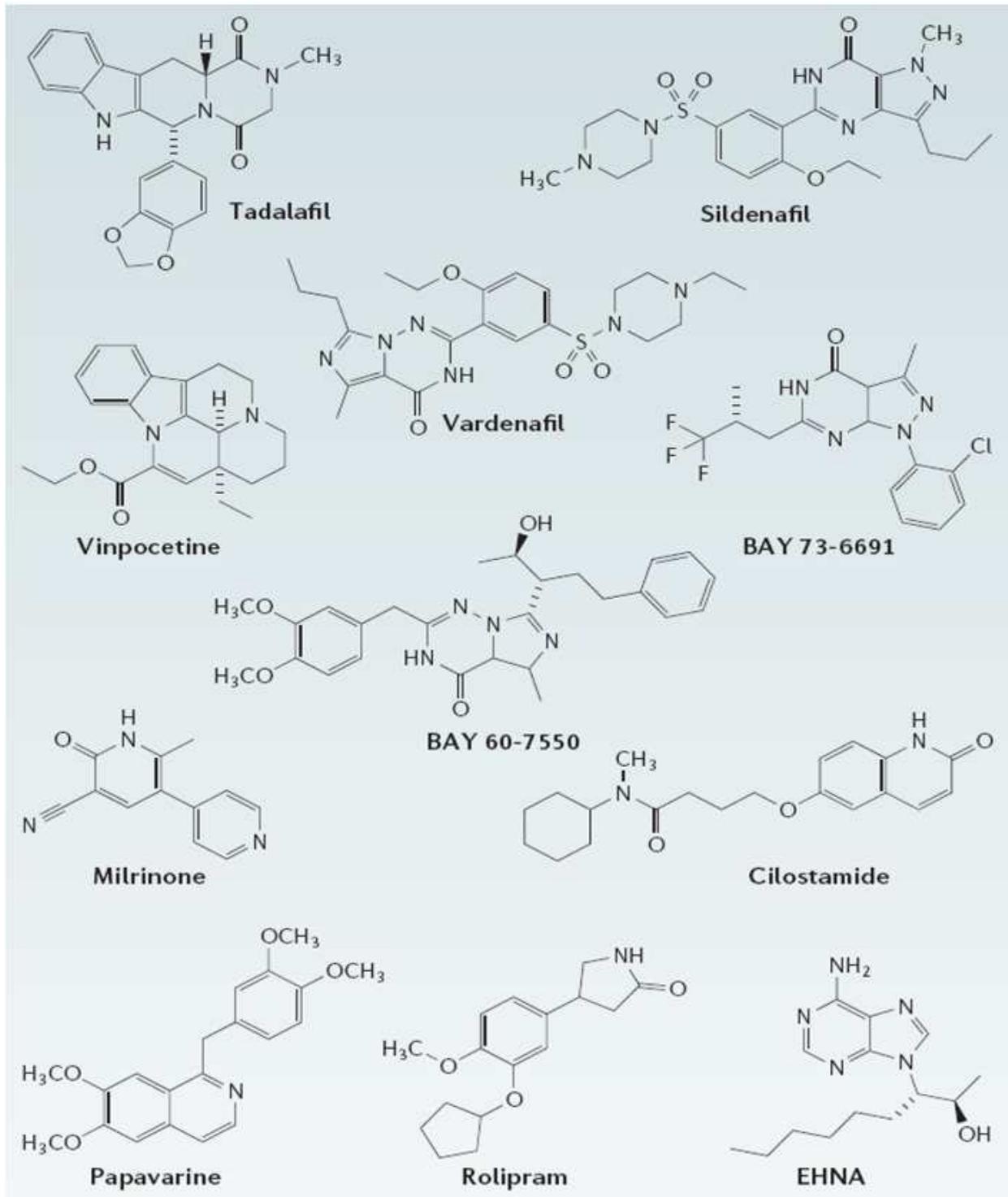


Fig. 2. Structures of selected examples of phosphodiesterase inhibitors. The figure shows various selective phosphodiesterase (PDE) inhibitors mentioned in this chapter. Of these, the PDE5 inhibitors sildenafil, vardenafil and tadalafil have been approved for treatment of erectile dysfunction. Sildenafil 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 tadalafil) 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 μ M 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.

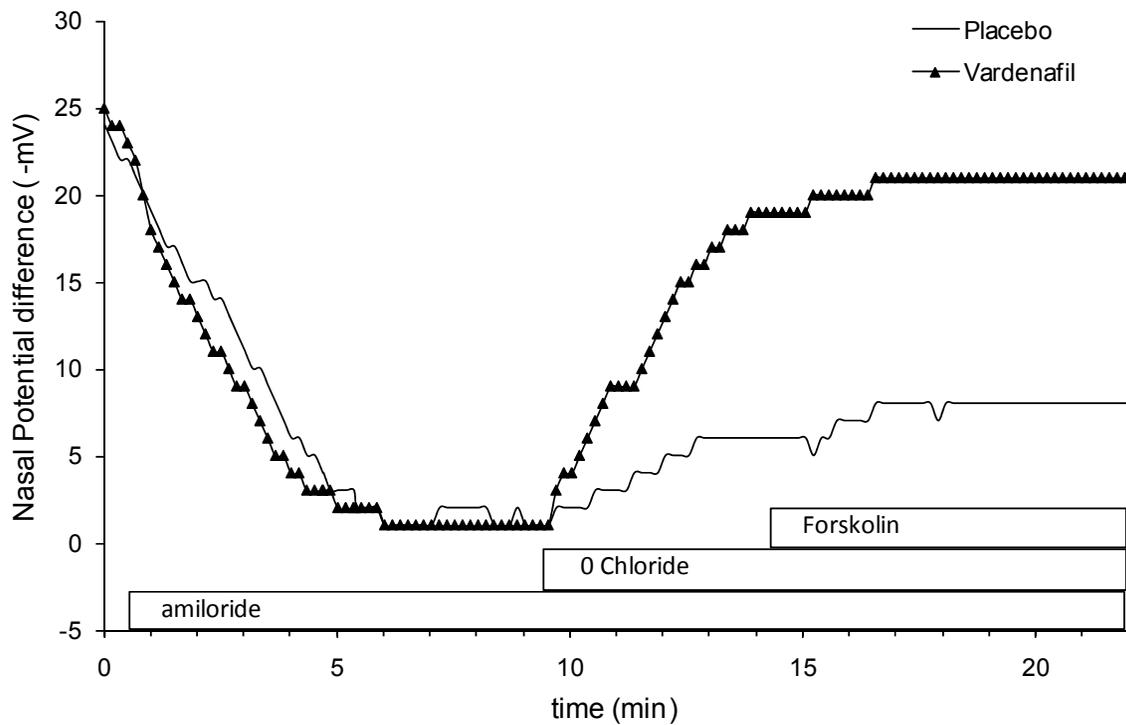


Fig. 3. Influence of vardenafil (24h after a single therapeutic dose) on ion transport evaluated by the nasal potential difference (PD) in F508del-CF mice. Chloride conductance in response to perfusion of the nasal mucosa with a solution without chloride and to forskolin is dramatically increased as compared to placebo-treated CF mice.

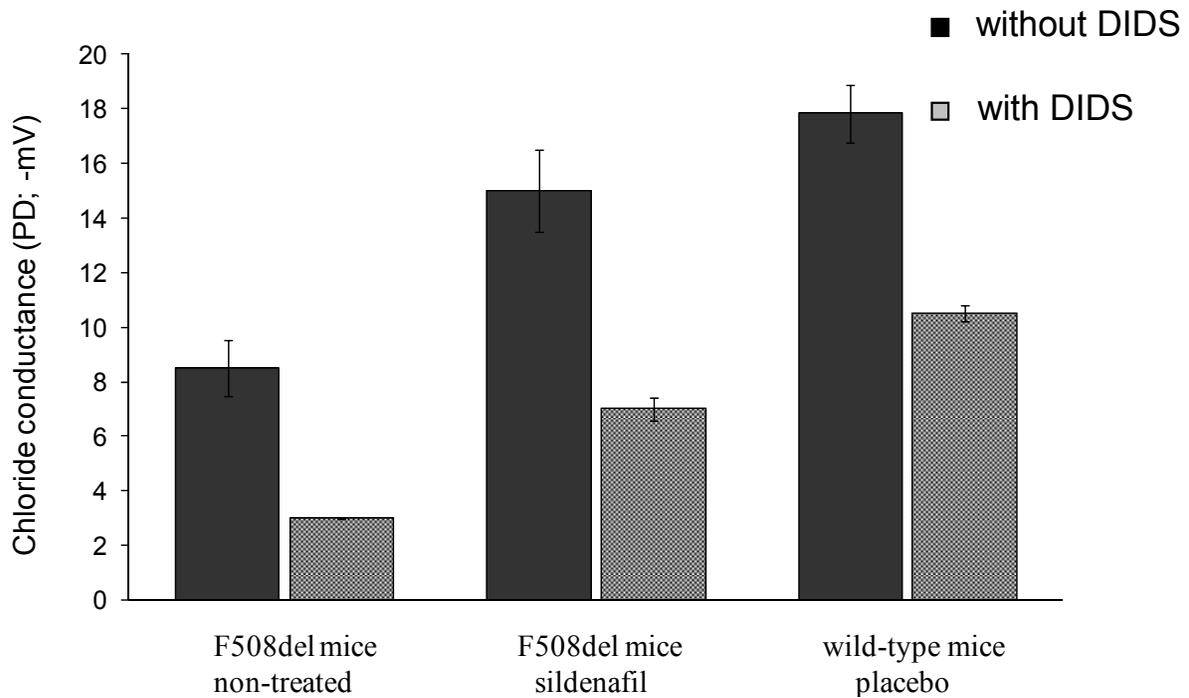


Fig. 4. Influence of sildenafil on Cftr -dependent chloride conductance evaluated by the nasal potential difference (PD) in the presence or the absence of DIDS, an inhibitor of alternative chloride conductance. Increased DIDS-insensitive conductance after sildenafil treatment reflects activation of Cftr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

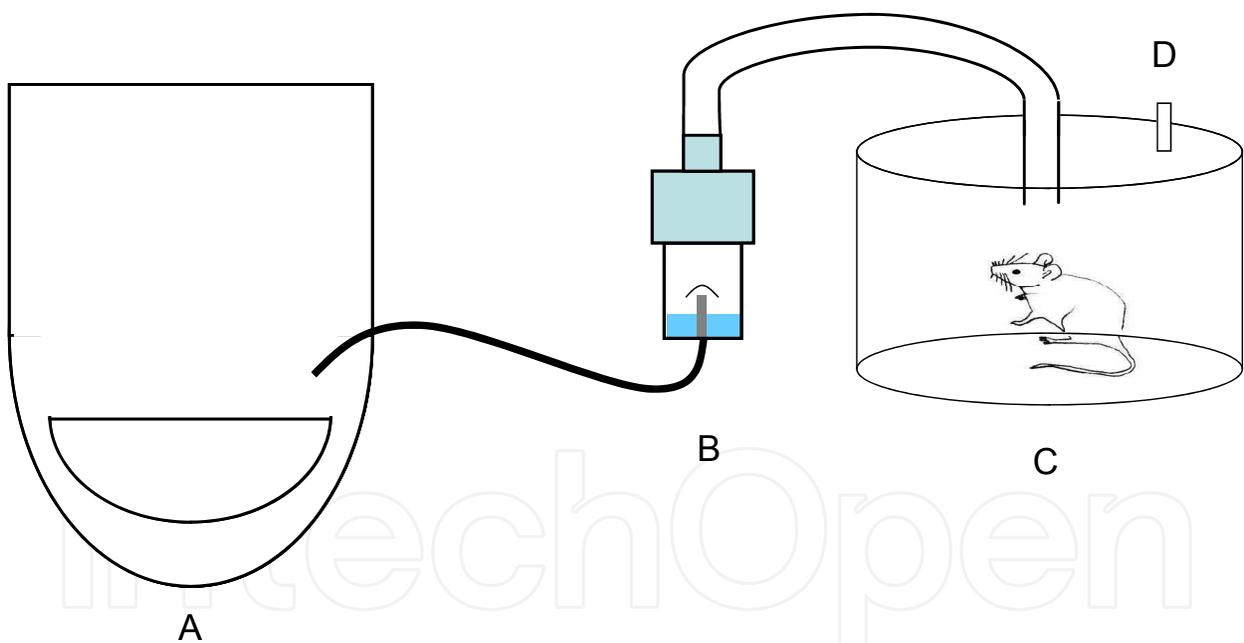


Fig. 5. Schematic representation of the whole-body immersion inhalation chamber setup we developed for a single mouse. (A) compressor, (B) nebulizer, (C) inhalation chamber with (D) expiratory gate.

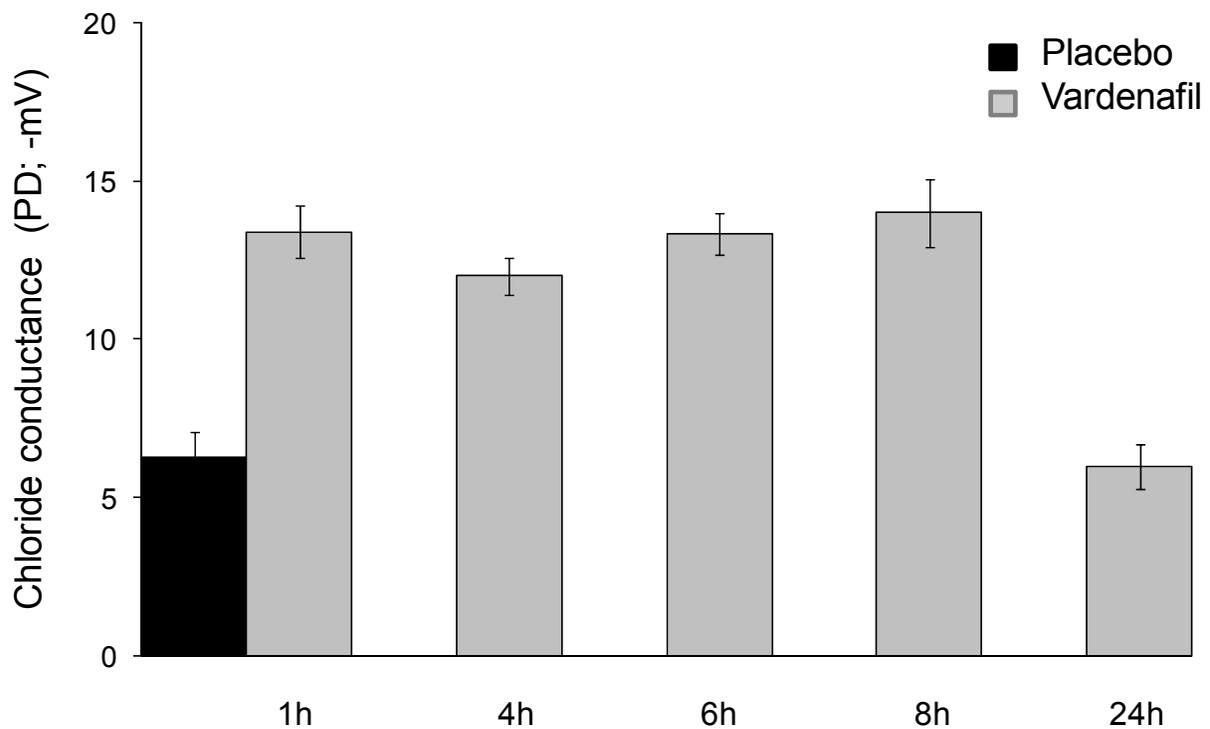


Fig. 6. Duration of the correcting effect of inhaled vardenafil on chloride conductance, evaluated by nasal potential difference (PD) in F508del-CF mice 1, 4, 6, 8 and 24h after a single nebulisation with placebo or with vardenafil. The correcting effect of vardenafil lasts at least 8 h after inhalation.

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 morbidity and 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 Cfr 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).

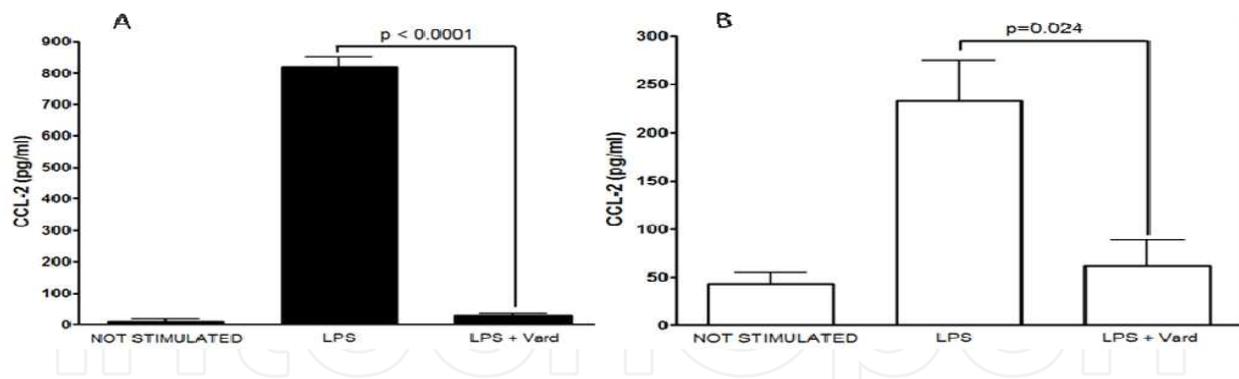


Fig. 7. Anti-inflammatory effect of *in vivo* treatment, by intraperitoneal injection, of a single therapeutic dose of vardenafil (vard) to F508del-CF (A) and wild-type (B) mice on the lipopolysaccharide (LPS of *P. aeruginosa*) induced inflammatory response. Biosynthesis of CCL-2 is significantly reduced 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. Acknowledgements

TL is an associate researcher with the Fonds de la Recherche Scientifique Médicale (FRSM). BL is a PhD fellow with the Fonds Spéciaux de Recherche (FSR; Université catholique de Louvain). SN is a postdoctoral fellow with the FSR and Marie Curie Actions of the European Commission. We thank Gregory Reychler for his assistance in the development of the inhalation chamber setup. Supported by grants of the French CF Association (Vaincre la Mucoviscidose), the FRSM, FSR and the Foundation St Luc (St Luc University Hospital and Université catholique de Louvain).

10. References

- [1] Kerem B, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, Buchwald M, Tsui LC (1989) Identification of the cystic fibrosis gene: Genetic analysis. *Science* 245:1073-1080.
- [2] Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, Lok S, Plavsic N, Chou JL, et al (1989). Identification of the cystic fibrosis gene: Cloning and characterization of complementary DNA. *Science* 245:1066-1073.
- [3] Rowe SM, Miller S, Sorscher EJ (2005) Cystic fibrosis. *N Engl J Med* 352:1992-2001.
- [4] Davis PB (2006) Cystic fibrosis since 1938. *Am J Respir Crit Care Med* 173:475-482.
- [5] Lukacs GL, Mohamed A, Kartner N, Chang XB, Riordan JR, Grinstein S (1994). Conformational maturation of CFTR but not its mutant counterpart (Delta F508) occurs in the endoplasmic reticulum and requires atp. *EMBO J* 13:6076-6086.
- [6] Amaral MD (2004) CFTR and chaperones: processing and degradation. *J Mol Neurosci* 23:41-8.
- [7] Dormer RL, Harris CM, Clark Z, Pereira MM, Doull IJ, Norez C, Becq F, McPherson MA (2005) Sildenafil (Viagra) corrects DeltaF508-CFTR location in nasal epithelial cells from patients with cystic fibrosis. *Thorax* 60:55-59.
- [8] Robert R, Carlile GW, Pavel C, Liu N, Anjos SM, Liao J, Luo Y, Zhang D, Thomas DY, Hanrahan JW (2008) Structural analog of sildenafil identified as a novel corrector of the F508del-CFTR trafficking defect. *Mol Pharmacol* 73:478-489.
- [9] Lubamba B, Lecourt H, Lebacq J, Lebecque P, De Jonge H, Wallemacq P, Leal T (2008). Preclinical evidence that sildenafil and vardenafil activate chloride transport in cystic fibrosis. *Am J Respir Crit Care Med* 177:506-515.
- [10] Lubamba B, Lebacq J, Reychler G, Marbaix E, Wallemacq P, Lebecque P, Leal T (2011) Inhaled phosphodiesterase type 5 inhibitors restore chloride transport in cystic fibrosis mice. *Eur Respir J* 37:72-78.
- [11] Beavo JA, Rogers NL, Crofford OB, Hardman JG, Sutherland EW, Newman EV (1970) Effects of xanthine derivatives on lipolysis and on adenosine 3',5'-monophosphate phosphodiesterase activity. *Mol Pharmacol* 6:597-603.
- [12] Cheung WY (1970) Cyclic nucleotide phosphodiesterase. *Adv Biochemical Psychopharmacol* 3:51-65.
- [13] Conti M (2000) Phosphodiesterases and cyclic nucleotide signaling in endocrine cells. *Mol Endocrinol* 14:1317-1327.
- [14] Soderling SH, Beavo JA (2000) Regulation of cAMP and cGMP signaling: new phosphodiesterases and new functions. *Curr Opin Cell Biol* 12:174-179
- [15] Francis SH, Turko IV, Corbin JD (2001) Cyclic nucleotide phosphodiesterases: relating structure and function. *Prog Nucleic Acid Res Mol Biol* 65:1-52.

- [16] Mehats C, Andersen CB, Filopanti M, Jin SL, Conti M (2002) Cyclic nucleotide phosphodiesterases and their role in endocrine cell signaling. *Trends Endocrinol Metabol* 13:29-35.
- [17] Xu RX, Rocque WJ, Lambert MH, Vanderwall DE, Luther MA, Nolte RT (2004) Crystal structures of the catalytic domain of phosphodiesterase 4B complexed with AMP, 8-Br-AMP, and rolipram. *J Mol Biol* 337:355-365.
- [18] Zhang HT, Zhao Y, Huang Y, Dorairaj NR, Chandler LJ, O'Donnell JM (2004) Inhibition of the phosphodiesterase 4 (PDE4) enzyme reverses memory deficits produced by infusion of the MEK inhibitor U0126 into the CA1 subregion of the rat hippocampus. *Neuropsychopharmacology* 29:1432-1439.
- [19] Yan C, Zhao AZ, Bentley JK, Loughney K, Ferguson K, Beavo JA (1995) Molecular cloning and characterization of a calmodulin-dependent phosphodiesterase enriched in olfactory sensory neurons. *Proc Natl Acad Sci USA* 92:9677-9681.
- [20] Loughney K, Martins TJ, Harris EA, Sadhu K, Hicks JB, Sonnenburg WK, Beavo JA, Ferguson K (1996) Isolation and characterization of cDNAs corresponding to two human calcium, calmodulin-regulated, 3',5'-cyclic nucleotide phosphodiesterases. *J Biol Chem* 271:796-806.
- [21] Yu SM, Hung LM, Lin CC (1997) cGMP-elevating agents suppress proliferation of vascular smooth muscle cells by inhibiting the activation of epidermal growth factor signaling pathway. *Circulation* 95:1269-1277.
- [22] Rosman GJ, Martins TJ, Sonnenburg WK, Beavo JA, Ferguson K, Loughney K (1997) Isolation and characterization of human cDNAs encoding a cGMP-stimulated 3',5'-cyclic nucleotide phosphodiesterase. *Gene* 191:89-95.
- [23] Rivet-Bastide M, Vandecasteele G, Hatem S, Verde I, Benardeau A, Mercadier JJ, Fischmeister R (1997) cGMP-stimulated cyclic nucleotide phosphodiesterase regulates the basal calcium current in human atrial myocytes. *J Clin Invest* 99:2710-2718.
- [24] Sadhu K, Hensley K, Florio VA, Wolda SL (1999) Differential expression of the cyclic GMP-stimulated phosphodiesterase PDE2A in human venous and capillary endothelial cells. *J Histochem Cytochem* 47:895-906.
- [25] Palmer D, Maurice DH (2000) Dual expression and differential regulation of phosphodiesterase 3A and phosphodiesterase 3B in human vascular smooth muscle: implications for phosphodiesterase 3 inhibition in human cardiovascular tissues. *Mol Pharmacol* 58:247-252.
- [26] Tenor H, Hatzelmann A, Kupferschmidt R, Stanciu L, Djukanovic R, Schudt C, Wendel A, Church MK, Shute JK (1995) Cyclic nucleotide phosphodiesterase isoenzyme activities in human alveolar macrophages. *Clin Exp Allergy* 25:625-633.
- [27] Tenor H, Hatzelmann A, Wendel A, Schudt C (1995) Identification of phosphodiesterase IV activity and its cyclic adenosine monophosphate-dependent up-regulation in a human keratinocyte cell line (HaCaT). *J Invest Dermatol* 105:70-74.
- [28] Tenor H, Stanciu L, Schudt C, Hatzelmann A, Wendel A, Djukanovic R, Church MK, Shute JK (1995) Cyclic nucleotide phosphodiesterases from purified human CD4+ and CD8+ T lymphocytes. *Clin Exp Allergy* 25:616-624.
- [29] Hamet P, Coquil JF (1978) Cyclic GMP binding and cyclic GMP phosphodiesterase in rat platelets. *J Cyclic Nucleotide Res* 4:281-290.

- [30] Coquil JF, Franks DJ, Wells JN, Dupuis M, Hamet P (1980) Characteristics of a new binding protein distinct from the kinase for guanosine 3':5'-monophosphate in rat platelets. *Biochim Biophys Acta* 631:148-165.
- [31] Francis SH, Corbin JD (1988) Purification of cGMP-binding protein phosphodiesterase from rat lung. *Meth Enzymol* 159:722-729.
- [32] Francis SH, Lincoln TM, Corbin JD (1980) Characterization of a novel cGMP binding protein from rat lung. *J Biol Chem* 255:620-626.
- [33] Sebkhi A, Strange JW, Phillips SC, Wharton J, Wilkins MR (2003) Phosphodiesterase type 5 as a target for the treatment of hypoxia-induced pulmonary hypertension. *Circulation* 107:3230-3235.
- [34] Takimoto E, Belardi D, Tocchetti CG, Vahebi S, Cormaci G, Ketner EA, Moens AL, Champion HC, Kass DA (2007) Compartmentalization of cardiac beta-adrenergic inotropy modulation by phosphodiesterase type 5. *Circulation* 115:2159-2167.
- [35] Zhang X, Feng Q, Cote RH (2005) Efficacy and selectivity of phosphodiesterase-targeted drugs in inhibiting photoreceptor phosphodiesterase (PDE6) in retinal photoreceptors. *Invest Ophthalmol Vis Sci* 46:3060-3066.
- [36] Smith SJ, Brookes-Fazakerley S, Donnelly LE, Barnes PJ, Barnette MS, Giembycz MA (2003) Ubiquitous expression of phosphodiesterase 7A in human proinflammatory and immune cells. *Am J Physiol* 284:L279-289.
- [37] Wang P, Wu P, Egan RW, Billah MM (2001) Human phosphodiesterase 8A splice variants: cloning, gene organization, and tissue distribution. *Gene* 280:183-194.
- [38] Hayashi M, Kita K, Ohashi Y, Aihara E, Takeuchi K (2007) Phosphodiesterase isozymes involved in regulation of HCO₃⁻ secretion in isolated mouse duodenum in vitro. *Biochem Pharmacol* 74:1507-1513.
- [39] Kobayashi T, Gamanuma M, Sasaki T, Yamashita Y, Yuasa K, Kotera J, Omori K (2003) Molecular comparison of rat cyclic nucleotide phosphodiesterase 8 family: unique expression of PDE8B in rat brain. *Gene* 319:21-31.
- [40] Perez-Torres S, Cortes R, Tolnay M, Probst A, Palacios JM, Mengod G (2003) Alterations on phosphodiesterase type 7 and 8 isozyme mRNA expression in Alzheimer's disease brains examined by in situ hybridization. *Exp Neurol* 182:322-334.
- [41] Glavas NA, Ostenson C, Schaefer JB, Vasta V, Beavo JA (2001) T cell activation up-regulates cyclic nucleotide phosphodiesterases 8A1 and 7A3. *Proc Natl Acad Sci USA* 98:6319-6324.
- [42] Dong H, Osmanova V, Epstein PM, Brocke S (2006) Phosphodiesterase 8 (PDE8) regulates chemotaxis of activated lymphocytes. *Biochem Biophys Res Commun* 345:713-719.
- [43] Vasta V, Shimizu-Albergine M, Beavo JA (2006) Modulation of Leydig cell function by cyclic nucleotide phosphodiesterase 8A. *Proc Natl Acad Sci USA* 103:19925-19930.
- [44] Dov A, Abramovitch E, Warwar N, Nesher R (2008) Diminished phosphodiesterase-8B potentiates biphasic insulin response to glucose. *Endocrinology* 149:741-748.
- [45] Soderling SH, Bayuga SJ, Beavo JA (1998) Cloning and characterization of a cAMP-specific cyclic nucleotide phosphodiesterase. *Proc Natl Acad Sci USA* 95:8991-8996.
- [46] Soderling SH, Bayuga SJ, Beavo JA (1998) Identification and characterization of a novel family of cyclic nucleotide phosphodiesterases. *J Biol Chem* 273:15553-15558.
- [47] Soderling SH, Bayuga SJ, Beavo JA (1999) Isolation and characterization of a dual-substrate phosphodiesterase gene family: PDE10A. *Proc Natl Acad Sci USA* 96:7071-7076.

- [48] Fujishige K, Kotera J, Michibata H, Yuasa K, Takebayashi S, Okumura K, Omori K (1999) Cloning and characterization of a novel human phosphodiesterase that hydrolyzes both cAMP and cGMP (PDE10A). *J Biol Chem* 274:18438-18445.
- [49] Loughney K, Snyder PB, Uher L, Rosman GJ, Ferguson K, Florio VA (1999) Isolation and characterization of PDE10A, a novel human 3', 5'-cyclic nucleotide phosphodiesterase. *Gene* 234:109-117.
- [50] Hebb AL, Robertson HA, Denovan-Wright EM (2004) Striatal phosphodiesterase mRNA and protein levels are reduced in Huntington's disease transgenic mice prior to the onset of motor symptoms. *Neuroscience* 123:967-981.
- [51] Fawcett L, Baxendale R, Stacey P, McGrouther C, Harrow I, Soderling S, Hetman J, Beavo JA, Phillips SC (2000) Molecular cloning and characterization of a distinct human phosphodiesterase gene family: PDE11A. *Proc Natl Acad Sci USA* 97:3702-3707.
- [52] Hetman JM, Soderling SH, Glavas NA, Beavo JA (2000) Cloning and characterization of PDE7B, a cAMP-specific phosphodiesterase. *Proc Natl Acad Sci USA* 97:472-476.
- [53] Weeks JL 2nd, Zoraghi R, Francis SH, Corbin JD (2007) N-Terminal domain of phosphodiesterase-11A4 (PDE11A4) decreases affinity of the catalytic site for substrates and tadalafil, and is involved in oligomerization. *Biochemistry* 46:10353-10364.
- [54] Bender AT, Beavo JA (2006) Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. *Pharmacol Rev* 58:488-520.
- [55] Bhatt-Mehta V, Schumacher RE. Treatment of apnea of prematurity. *Paediatr Drugs* 2003;5:195-210.
- [56] Barnes PJ (2003) Theophylline: new perspectives for an old drug. *Am J Respir Crit Care Med* 167:813-818.
- [57] Barnes PJ (2003) Therapy of chronic obstructive pulmonary disease. *Pharmacol Ther* 97:87-94.
- [58] Barnes PJ (200) Theophylline in chronic obstructive pulmonary disease: new horizons. *Proc Am Thorac Soc* 2:334-339.
- [59] Barnes PJ, Stockley RA (2005) COPD: current therapeutic interventions and future approaches. *Eur Respir J* 25:1084-1106.
- [60] Muller CE, Jacobson KA (2011) Xanthines as adenosine receptor antagonists. *Handb Exp Pharmacol*:151-199.
- [61] Sullivan M, Egerton M, Shakur Y, Marquardsen A, Houslay MD (1994) Molecular cloning and expression, in both COS-1 cells and *S. cerevisiae*, of a human cytosolic type-IVA, cyclic AMP specific phosphodiesterase (hPDE-IVA-h6.1). *Cell Signal* 6:793-812.
- [62] Guerreiro S, Toulorge D, Hirsch E, Marien M, Sokoloff P, Michel PP (2008) Paraxanthine, the primary metabolite of caffeine, provides protection against dopaminergic cell death via stimulation of ryanodine receptor channels. *Mol Pharmacol* 74:980-989.
- [63] Wells JN, Wu YJ, Baird CE, Hardman JG (1975) Phosphodiesterases from porcine coronary arteries: inhibition of separated forms by xanthines, papaverine, and cyclic nucleotides. *Mol Pharmacol* 11:775-783.
- [64] Suvarna NU, O'Donnell JM (2002) Hydrolysis of N-methyl-D-aspartate receptor-stimulated cAMP and cGMP by PDE4 and PDE2 phosphodiesterases in primary neuronal cultures of rat cerebral cortex and hippocampus. *J Pharmacol Exp Ther* 302:249-256.

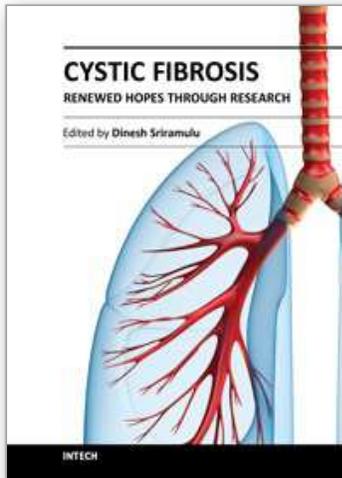
- [65] Podzuweit T, Nennstiel P, Muller A (1995) Isozyme selective inhibition of cGMP-stimulated cyclic nucleotide phosphodiesterases by erythro-9-(2-hydroxy-3-nonyl) adenine. *Cell Signal* 7:733-738.
- [66] Repaske DR, Swinnen JV, Jin SL, Van Wyk JJ, Conti M (1992) A polymerase chain reaction strategy to identify and clone cyclic nucleotide phosphodiesterase cDNAs. Molecular cloning of the cDNA encoding the 63-kDa calmodulin-dependent phosphodiesterase. *J Biol Chem* 267:18683-18688.
- [67] Boess FG, Hendrix M, van der Staay FJ, Erb C, Schreiber R, van Staveren W, de Vente J, Prickaerts J, Blokland A, Koenig G (2004) Inhibition of phosphodiesterase 2 increases neuronal cGMP, synaptic plasticity and memory performance. *Neuropharmacology* 47:1081-1092.
- [68] Rutten K, Van Donkelaar EL, Ferrington L, Blokland A, Bollen E, Steinbusch HW, Kelly PA, Prickaerts JH (2009) Phosphodiesterase inhibitors enhance object memory independent of cerebral blood flow and glucose utilization in rats. *Neuropsychopharmacology* 34:1914-1925.
- [69] Reed TM, Repaske DR, Snyder GL, Greengard P, Vorhees CV (2002) Phosphodiesterase 1B knock-out mice exhibit exaggerated locomotor hyperactivity and DARPP-32 phosphorylation in response to dopamine agonists and display impaired spatial learning. *J Neurosci* 22:5188-5197.
- [70] Truss MC, Stief CG, Uckert S, Becker AJ, Wefer J, Schultheiss D, Jonas U (2001) Phosphodiesterase 1 inhibition in the treatment of lower urinary tract dysfunction: from bench to bedside. *World J Urol* 19:344-350.
- [71] Medina AE, Krahe TE, Ramoa AS (2006) Restoration of neuronal plasticity by a phosphodiesterase type 1 inhibitor in a model of fetal alcohol exposure. *J Neurosci* 26:1057-1060.
- [72] Menniti FS, Faraci WS, Schmidt CJ (2006) Phosphodiesterases in the CNS: targets for drug development. *Nat Rev Drug Discov* 5:660-670.
- [73] Filgueiras CC, Krahe TE, Medina AE (2010) Phosphodiesterase type 1 inhibition improves learning in rats exposed to alcohol during the third trimester equivalent of human gestation. *Neurosci Lett* 473:202-207.
- [74] Jeon KI, Xu X, Aizawa T, Lim JH, Jono H, Kwon DS, Abe J, Berk BC, Li JD, Yan C (2010) Vinpocetine inhibits NF-kappaB-dependent inflammation via an IKK-dependent but PDE-independent mechanism. *Proc Natl Acad Sci USA* 107:9795-9800.
- [75] Medina AE (2010) Vinpocetine as a potent antiinflammatory agent. *Proc Natl Acad Sci USA* 107:9921-9922.
- [76] Vandecasteele G, Verde I, Rucker-Martin C, Donzeau-Gouge P, Fischmeister R (2001) Cyclic GMP regulation of the L-type Ca(2+) channel current in human atrial myocytes. *J Physiol* 533:329-340.
- [77] Shin DD, Brandimarte F, De Luca L, Sabbah HN, Fonarow GC, Filippatos G, Komajda M, Gheorghiade M (2007) Review of current and investigational pharmacologic agents for acute heart failure syndromes. *Am J Cardiol* 99:4A-23A.
- [78] Nohria A, Tsang SW, Fang JC, Lewis EF, Jarcho JA, Mudge GH, Stevenson LW (2003) Clinical assessment identifies hemodynamic profiles that predict outcomes in patients admitted with heart failure. *J Am Coll Cardiol* 41:1797-1804.
- [79] Carev M, Bulat C, Karanovic N, Lojpur M, Jercic A, Nenadic D, Marovich Z, Husedzinovic I, Letica D (2010) Combined usage of inhaled and intravenous milrinone in pulmonary hypertension after heart valve surgery. *Coll Antropol* 34:1113-1117.

- [80] Essayan DM (2001) Cyclic nucleotide phosphodiesterases. *J Allergy Clin Immunol* 108:671-680.
- [81] Toward TJ, Smith N, Broadley KJ (2004) Effect of phosphodiesterase-5 inhibitor, sildenafil (Viagra), in animal models of airways disease. *Am J Respir Crit Care Med* 169:227-234.
- [82] Scott AI, Perini AF, Shering PA, Whalley LJ (1991) In-patient major depression: is rolipram as effective as amitriptyline? *Eur J Clin Pharmacol* 40:127-129.
- [83] O'Byrne PM, Gauvreau G (2009) Phosphodiesterase-4 inhibition in COPD. *Lancet* 374:665-667.
- [84] Ghofrani HA, Osterloh IH, Grimminger F (2006) Sildenafil: from angina to erectile dysfunction to pulmonary hypertension and beyond. *Nature Rev Drug Discov* 5:689-702.
- [85] Corbin JD, Beasley A, Blount MA, Francis SH (2005) High lung PDE5: a strong basis for treating pulmonary hypertension with PDE5 inhibitors. *Biochem Biophys Res Commun* 334:930-938.
- [86] Wharton J, Strange JW, Moller GM, Growcott EJ, Ren X, Franklyn AP, Phillips SC, Wilkins MR (2005) Antiproliferative effects of phosphodiesterase type 5 inhibition in human pulmonary artery cells. *Am J Respir Crit Care Med* 172:105-113.
- [87] Moncada S, Martin JF (1993) Evolution of nitric oxide. *Lancet* 341:1511.
- [88] Prickaerts J, van Staveren WC, Sik A, Markerink-van Ittersum M, Niewohner U, van der Staay FJ, Blokland A, de Vente J (2002) Effects of two selective phosphodiesterase type 5 inhibitors, sildenafil and vardenafil, on object recognition memory and hippocampal cyclic GMP levels in the rat. *Neuroscience* 113:351-361.
- [89] Baratti CM, Boccia MM (1999) Effects of sildenafil on long-term retention of an inhibitory avoidance response in mice. *Behav Pharmacol* 10:731-737.
- [90] Gardner C, Robas N, Cawkill D, Fidock M (2000) Cloning and characterization of the human and mouse PDE7B, a novel cAMP-specific cyclic nucleotide phosphodiesterase. *Biochem Biophys Res Commun* 272:186-192.
- [91] Sasaki T, Kotera J, Yuasa K, Omori K (2000) Identification of human PDE7B, a cAMP-specific phosphodiesterase. *Biochem Biophys Res Commun* 271:575-583.
- [92] Pitts WJ, Vaccaro W, Huynh T, Leftheris K, Roberge JY, Barbosa J, Guo J, Brown B, Watson A, Donaldson K, Starling GC, Kiener PA, Poss MA, Dodd JH, Barrish JC (2004) Identification of purine inhibitors of phosphodiesterase 7 (PDE7). *Bioorg Med Chem Lett* 14:2955-2958.
- [93] Vergne F, Bernardelli P, Lorthiois E, Pham N, Proust E, Oliveira C, Mafroud AK, Royer F, Wrigglesworth R, Schellhaas J, Barvian M, Moreau F, Idrissi M, Tertre A, Bertin B, Coupe M, Berna P, Soulard P (2004) Discovery of thiazoles as a novel structural class of potent and selective PDE7 inhibitors. Part 1: design, synthesis and structure-activity relationship studies. *Bioorg Med Chem Lett* 14:4607-4613.
- [94] Zhang L, Murray F, Zahno A, Kanter JR, Chou D, Suda R, Fenlon M, Rassenti L, Cottam H, Kipps TJ, Insel PA (2008) Cyclic nucleotide phosphodiesterase profiling reveals increased expression of phosphodiesterase 7B in chronic lymphocytic leukemia. *Proc Natl Acad Sci USA* 105:19532-19537.
- [95] Vang AG, Ben-Sasson SZ, Dong H, Kream B, DeNinno MP, Claffey MM, Housley W, Clark RB, Epstein PM, Brocke S (2010) PDE8 regulates rapid T cell adhesion and proliferation independent of ICER. *PLoS One* 5:e12011.

- [96] Tsai LC, Shimizu-Albergine M, Beavo JA (2011) The high affinity cAMP-specific phosphodiesterase 8B (PDE8B) controls steroidogenesis in the mouse adrenal gland. *Mol Pharmacol* 79:639-648.
- [97] Antoni FA (2000) Molecular diversity of cyclic AMP signalling. *Front Neuroendocrinol* 21:103-132.
- [98] Chappe V, Mettey Y, Vierfond JM, Hanrahan JW, Gola M, Verrier B, Becq F (1998) Structural basis for specificity and potency of xanthine derivatives as activators of the CFTR chloride channel. *Br J Pharmacol* 123:683-693.
- [99] Drumm ML, Wilkinson DJ, Smit LS, Worrell RT, Strong TV, Frizzell RA, Dawson DC, Collins FS (1991) Chloride conductance expressed by delta F508 and other mutant CFTRs in *Xenopus* oocytes. *Science* 254:1797-1799.
- [100] Grubb B, Lazarowski E, Knowles M, Boucher R (1993) Isobutylmethylxanthine fails to stimulate chloride secretion in cystic fibrosis airway epithelia. *Am J Respir Cell Mol Biol* 8:454-460.
- [101] Haws CM, Nepomuceno IB, Krouse ME, Wakelee H, Law T, Xia Y, Nguyen H, Wine JJ (1996) Delta F508-CFTR channels: kinetics, activation by forskolin, and potentiation by xanthines. *Am J Physiol* 270:C1544-1555.
- [102] Takeuchi K, Yagi K, Kato S, Ukawa H (1997) Roles of prostaglandin E-receptor subtypes in gastric and duodenal bicarbonate secretion in rats. *Gastroenterology* 113:1553-1559.
- [103] Aoi M, Aihara E, Nakashima M, Takeuchi K (2004) Participation of prostaglandin E receptor EP4 subtype in duodenal bicarbonate secretion in rats. *Am J Physiol Gastrointest Liver Physiol* 287:G96-103.
- [104] Kelley TJ, Al-Nakkash L, Drumm ML (1995) CFTR-mediated chloride permeability is regulated by type III phosphodiesterases in airway epithelial cells. *Am J Respir Cell Mol Biol* 13:657-664.
- [105] Kelley TJ, Al-Nakkash L, Cotton CU, Drumm ML (1996) Activation of endogenous deltaF508 cystic fibrosis transmembrane conductance regulator by phosphodiesterase inhibition. *J Clin Invest* 98:513-520.
- [106] Kelley TJ, Thomas K, Milgram LJ, Drumm ML (1997) In vivo activation of the cystic fibrosis transmembrane conductance regulator mutant deltaF508 in murine nasal epithelium. *Proc Natl Acad Sci USA* 94:2604-2608.
- [107] Smith SN, Middleton PG, Chadwick S, Jaffe A, Bush KA, Rolleston S, Farley R, Delaney SJ, Wainwright B, Geddes DM, Alton EW (1999) The in vivo effects of milrinone on the airways of cystic fibrosis mice and human subjects. *Am J Respir Cell Mol Biol* 20:129-134.
- [108] Hogan DL, Crombie DL, Isenberg JI, Svendsen P, Schaffalitzky de Muckadell OB, Ainsworth MA (1997) CFTR mediates cAMP- and Ca²⁺-activated duodenal epithelial HCO₃⁻ secretion. *Am J Physiol* 272:G872-878.
- [109] O'Grady SM, Jiang X, Maniak PJ, Birmachu W, Scribner LR, Bulbulian B, Gullikson GW (2002) Cyclic AMP-dependent Cl secretion is regulated by multiple phosphodiesterase subtypes in human colonic epithelial cells. *J Membr Biol* 185:137-144.
- [110] Hayashi M, Kita K, Ohashi Y, Aihara E, Takeuchi K (2007) Phosphodiesterase isozymes involved in regulation of HCO₃⁻ secretion in isolated mouse duodenum in vitro. *Biochem Pharmacol* 74:1507-1513.
- [111] McPherson MA, Pereira MM, Lloyd Mills C, Murray KJ, Dormer RL (1999) A cyclic nucleotide PDE5 inhibitor corrects defective mucin secretion in submandibular

- cells containing antibody directed against the cystic fibrosis transmembrane conductance regulator protein. *FEBS Lett* 464:48-52.
- [112] Ahn HS, Foster M, Cable M, Pitts BJ, Sybertz EJ (1991) Ca/CaM-stimulated and cGMP-specific phosphodiesterases in vascular and non-vascular tissues. *Adv Exp Med Biol* 308:191-197.
- [113] Milligan PA, Marshall SF, Karlsson MO (2002) A population pharmacokinetic analysis of sildenafil citrate in patients with erectile dysfunction. *Br J Clin Pharmacol* 53 Suppl 1:45S-52S.
- [114] Nichols DJ, Muirhead GJ, Harness JA (2002) Pharmacokinetics of sildenafil after single oral doses in healthy male subjects: absolute bioavailability, food effects and dose proportionality. *Br J Clin Pharmacol* 53 Suppl 1:5S-12S.
- [115] Muirhead GJ, Rance DJ, Walker DK, Wastall P (2002) Comparative human pharmacokinetics and metabolism of single-dose oral and intravenous sildenafil. *Br J Clin Pharmacol* 53 Suppl 1:13S-20S.
- [116] Burgess G, Hoogkamer H, Collings L, Dingemans J (2008) Mutual pharmacokinetic interactions between steady-state bosentan and sildenafil. *Eur J Clin Pharmacol* 64:43-50.
- [117] Klotz T, Sachse R, Heidrich A, Jockenhovel F, Rohde G, Wensing G, Horstmann R, Engelmann R (2001) Vardenafil increases penile rigidity and tumescence in erectile dysfunction patients: a RigiScan and pharmacokinetic study. *World J Urol* 19:32-39.
- [118] Gresser U, Gleiter CH (2002) Erectile dysfunction: comparison of efficacy and side effects of the PDE-5 inhibitors sildenafil, vardenafil and tadalafil--review of the literature. *Eur J Med Res* 7:435-446.
- [119] Stark S, Sachse R, Liedl T, Hensen J, Rohde G, Wensing G, Horstmann R, Schrott KM (2001) Vardenafil increases penile rigidity and tumescence in men with erectile dysfunction after a single oral dose. *Eur Urol* 40:181-188; discussion 189-190.
- [120] Ormrod D, Easthope SE, Figgitt DP (2002) Vardenafil. *Drugs Aging* 19:217-227.
- [121] Eardley I, Cartledge J (2002) Tadalafil (Cialis) for men with erectile dysfunction. *Int J Clin Pract* 56:300-304.
- [122] Bella AJ, Brock GB (2003) Tadalafil in the treatment of erectile dysfunction. *Curr Urol Rep* 4:472-478.
- [123] Staab A, Tillmann C, Forgue ST, Mackie A, Allerheiligen SR, Rapado J, Troconiz IF (2004) Population dose-response model for tadalafil in the treatment of male erectile dysfunction. *Pharm Res* 21:1463-1470.
- [124] Brock GB (2003) Tadalafil: a new agent for erectile dysfunction. *Can J Urol* 10 Suppl 1:17-22.
- [125] Porst H, Padma-Nathan H, Giuliano F, Anglin G, Varanese L, Rosen R (2003) Efficacy of tadalafil for the treatment of erectile dysfunction at 24 and 36 hours after dosing: a randomized controlled trial. *Urology* 62:121-125; discussion 125-126.
- [126] Curran M, Keating G (2003) Tadalafil. *Drugs* 63:2203-2212; discussion 2213-2214.
- [127] Pedemonte N, Lukacs GL, Du K, Caci E, Zegarra-Moran O, Galiotta LJ, Verkman AS (2005) Small-molecule correctors of defective DeltaF508-CFTR cellular processing identified by high-throughput screening. *J Clin Invest* 115:2564-2571.
- [128] Van Goor F, Straley KS, Cao D, Gonzalez J, Hadida S, Hazlewood A, Joubran J, Knapp T, Makings LR, Miller M, Neuberger T, Olson E, Panchenko V, Rader J, Singh A, Stack JH, Tung R, Grootenhuys PD, Negulescu P (2006) Rescue of DeltaF508-CFTR trafficking and gating in human cystic fibrosis airway primary cultures by small molecules. *Am J Physiol Lung Cell Mol Physiol* 290:L1117-1130.

- [129] Carlile GW, Robert R, Zhang D, Teske KA, Luo Y, Hanrahan JW, Thomas DY (2007) Correctors of protein trafficking defects identified by a novel high-throughput screening assay. *Chem Biochem* 8:1012-1020.
- [130] van Doorninck JH, French PJ, Verbeek E, Peters RH, Morreau H, Bijman J, Scholte BJ (1995) A mouse model for the cystic fibrosis delta F508 mutation. *EMBO J* 14:4403-4411.
- [131] Lubamba B, Lebacq J, Lebecque P, Vanbever R, Leonard A, Wallemacq P, Leal T (2009) Airway delivery of low dose miglustat normalizes nasal potential difference in F508del cystic fibrosis mice. *Am J Respir Crit Care Med* 179:1022-8.
- [132] Sermet-Gaudelus I, De Boeck K, Casimir GJ, Vermeulen F, Leal T, Mogenet A, Roussel D, Fritsch J, Constantine S, Reha A, Hirawat S, Miller NL, Ajayi T, Elfring GL, Miller L (2010) Ataluren (PTC124) Induces CFTR Protein Expression and Activity in Children with Nonsense Mutation Cystic Fibrosis. *Am J Respir Crit Care Med* 182:1262-72.
- [133] Berry B, Altman P, Rowe J, Vaisman T (2009) Comparison of pharmacokinetics of vardenafil administered using an ultrasonic nebulizer for inhalation versus a single 10-mg oral tablet. *J Sex Med* July 28 [Epub ahead of print].
- [134] Dalby R, Suman J (2003) Inhalation therapy: technological milestones in asthma treatment. *Adv Drug Deliv Rev* 55: 779-791.
- [135] Legssyer R, Huaux F, Lebacq J, Delos M, Marbaix E, Lebecque P, Lison D, Scholte BJ, Wallemacq P, Leal T (2006) Azithromycin reduces spontaneous and induced inflammation in delta F508 cystic fibrosis mice. *Respir Res* 7:134.
- [136] Meyer M, Huaux F, Gavilanes X, van den Brûle S, Lebecque P, Lo Re S, Lison D, Scholte B, Wallemacq P, Leal T (2009) Azithromycin reduces exaggerated cytokine production by M1 alveolar macrophages in cystic fibrosis. *Am J Respir Cell Mol Biol* 41:590-602.
- [137] Gavilanes X, Huaux F, Meyer M, Lebecque P, Marbaix E, Lison D, Scholte B, Wallemacq P, Leal T (2009) Azithromycin fails to reduce increased expression of neutrophil-related cytokines in primary-cultured epithelial cells from cystic fibrosis mice. *J Cyst Fibros* 8:203-210.
- [138] Poschet JF, Timmins GS, Taylor-Cousar JL, Ornatowski W, Fazio J, Perkett E, Wilson KR, Yu HD, de Jonge HR, Deretic V (2007) Pharmacological modulation of cGMP levels by phosphodiesterase 5 inhibitors as a therapeutic strategy for treatment of respiratory pathology in cystic fibrosis. *Am J Physiol Lung Cell Mol Physiol* 293:L712-719.
- [139] Wang Y, Loo TW, Bartlett MC, Clarke DM (2007) Correctors promote maturation of cystic fibrosis transmembrane conductance regulator (CFTR)-processing mutants by binding to the protein. *J Biol Chem* 282:33247-33251.
- [140] Lubamba BA, Panin N, Wauthier S, Huaux F, Lison D, Lebecque P, Wallemacq P, Leal T (2010) Anti-inflammatory effect of vardenafil in CF lung disease. *Ped Pulmonol* 45(S33):308-309.



Cystic Fibrosis - Renewed Hopes Through Research

Edited by Dr. Dinesh Sriramulu

ISBN 978-953-51-0287-8

Hard cover, 550 pages

Publisher InTech

Published online 28, March, 2012

Published in print edition March, 2012

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.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Bob Lubamba, Barbara Dhooghe, Sabrina Noël and Teresinha Leal (2012). Pharmacological Potential of PDE5 Inhibitors for the Treatment of Cystic Fibrosis, Cystic Fibrosis - Renewed Hopes Through Research, Dr. Dinesh Sriramulu (Ed.), ISBN: 978-953-51-0287-8, InTech, Available from:

<http://www.intechopen.com/books/cystic-fibrosis-renewed-hopes-through-research/pharmacological-potential-of-pde5-inhibitors-for-the-treatment-of-cystic-fibrosis>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen