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Phosphodiesterase 3 and 4 Inhibition: Facing a Bright Future in Asthma Control

Jan Beute, Vincent Manganiello and Alex Klein

Abstract

A recent status on asthmaticus multiple case report by Beute demonstrated the beneficial effects of phosphodiesterase III (PDE3) and phosphodiesterase IV (PDE4) inhibition. This chapter reviews the possible underlying mechanisms, beside the known effect, for the beneficial effects of a mixed PDE3/4 inhibitor in allergic airway inflammation. Structural cells of the lung and immune system express PDE3 and 4. PDE3 and 4 inhibition have a number of consequences related to physical function and cytokine production. The most direct effect of PDE3 inhibition being relaxation of smooth muscle cells results in bronchodilation. However, PDE3 inhibition appears to go further than a mere inhibitory activity in bronchial smooth muscle. It also affects structural cells, and more importantly, it creates an improved barrier function in endothelial cells. PDE3 and 4 inhibition therefore strengthens the immune barrier; but in addition, it modifies the cells of the immune system itself, as these also express PDE3 and 4 activity, thus changing their function. All aspects of asthma-related pathophysiology seem to be affected by PDE3 and 4 inhibition. Clinical use of a mixed PDE3/4 inhibitor in respiratory diseases is currently limited to a few studies, including life-threatening asthma in which mixed PDE3/4 inhibition has a beneficial effect.

Keywords: PDE3, PDE4, allergic airway inflammation

1. Introduction

Asthma is an obstructive airway disease characterized by inflamed airways, structural and physiological abnormalities in the airways, and shortness of breath [1]. Important primary airway cells are alveolar cells, endothelial cells, and smooth muscle cells; and secondary cells...
are involved in regulation of innate and adoptive immunology. Conventional treatment with inhaled corticosteroids combined with beta-adrenergic agonists supports and induces smooth muscle relaxation to reopen the inflamed airways, relieves symptoms, supports inspirational and expirational flow, and reduces inflammation [2]. These treatment regimens were also used in the extreme severe cases of asthma like status asthmaticus and patients with bronchospasm, in some cases with only minimal effect. The treatment goal in these severe cases of acute asthma is the prompt relief of respiratory distress. The great benefit of a mixed PDE3/4 inhibitor, in these severe cases, is the induction of acute as well as long-lasting bronchodilator effects [3].

At the moment, there is no effective treatment for these severe asthmatic patients [4] and there are no clear, effective guidelines. Moreover, treatment of these patients is multidisciplinary, involving first aid physicians, intensive care physicians, anesthetists, and pulmonary physicians, requiring golden standards and treatment regimens per hospital for optimal results. There are still too many asthma deaths; numbers in US are up to nine cases per day and in the UK are up to over three cases per day. Presently, in the Netherlands, there are annually more than 60.

This review discusses the PDE3 gene family and PDE3 inhibition, traditionally used in acute, refractory heart failure. We discuss the benefits of combined PDE3 and 4 inhibition in status asthmaticus [3], and the possible mechanisms which may be responsible for these beneficial effects of PDE3 and 4 inhibition.

2. The PDE superfamily: important regulators of cyclic nucleotide signaling pathways and networks

Intracellular signaling via complicated regulatory networks plays a critical role during physiological cellular responses. cAMP and cGMP were the first molecules described as intracellular second messengers [5]. They regulate multiple intracellular targets, including protein kinase A and protein kinase G, guanine nucleotide exchange proteins activated by cAMP (Epacs), cyclic nucleotide-gated ion channels, and PDE activities [6]. Intracellular concentrations of cAMP and cGMP are regulated through their synthesis by adenylyl cyclases (ACs) and guanylyl cyclases and their degradation via cyclic nucleotide PDEs. Ten different ACs have been identified and classified into two groups [7]. The first group consists of transmembrane enzymes which are activated by different hormones, neurotransmitters, chemokines, and cytokines in the G-protein-coupled receptor cascade [8]. Another group of cytosolic ACs is regulated by bicarbonate and calcium ions [8]. Whereas, cytosolic ACs are all encoded by one gene, transmembrane ACs represent a group encoded by nine different genes [9].

The large PDE superfamily is comprised of 11 PDE gene families (PDE1–PDE11). They specifically hydrolyze cyclic nucleotides, and can be classified according to their primary structures, tissue expression, biochemical properties, regulation, and their sensitivity to different pharmacological agents [10]. By catalyzing the hydrolysis of cAMP and cGMP, PDEs regulate the intracellular concentrations of these critical second messengers, and consequently, their downstream signaling pathways and networks. PDEs also function as important regulators in the compartmentation of cyclic nucleotide signaling pathways and networks. Individual PDEs are targeted/recruited to specific intracellular locations, where they are incorporated.
into specific multiprotein regulatory complexes (“signalosomes”) through protein-protein interactions. By virtue of their localization to specific compartments, PDEs can thus regulate specific cyclic nucleotide signaling pathways [11].

### 3. PDE3 and PDE4

PDE3 is expressed in pulmonary structural cells and cells of the immune system. Lung structural cells, including smooth muscle cells, epithelial cells, and endothelial cells, express PDE3. PDE3A and B are encoded by two highly related and similarly organized genes on human chromosomes, 12p12 and 11p15 [12–14]. Both PDE3A and PDE3B hydrolyze cAMP and cGMP, with 4–10 times higher affinity (Vmax) for cAMP [15]. Biochemical and histochemical studies of the localization of PDE3 suggested that PDE3 was associated with the sarcoplasmic reticulum, Golgi endosome, and nuclear envelope in cardiac tissue [16]. PDE3 plays a major role in cardiac contraction by modulating cAMP-dependent phosphorylation of voltage-gated Ca\(^{2+}\) channels and Ca\(^{2+}\) entry [17]. In addition, recent studies with PDE3A and PDE3B KO mice indicate that PDE3A, not PDE3B, regulates basal contractility in mouse heart [18].

Kass et al. described one mechanism, whereby PDE3 might be functionally modulated by cGMP occupying the PDE3 catalytic site [19]. PDE3 binds both cAMP and cGMP at its catalytic site with high affinity, and endogenous cGMP, generated by NO-induced activation of guanyl cyclase, can function as a competitive inhibitor of hydrolysis of cAMP by PDE3 [20]. NO-induced cGMP/cAMP cross-talk, mediated via cGMP inhibition of cAMP hydrolysis by PDE3 which leads to increased levels of cAMP, is thought to mediate some of the effects of NO in inflammatory and lung structural cells. NO modulates pulmonary vascular tone, causing non-adrenergic-, non-cholinergic-mediated bronchodilation [21]. Overexpression of nitric oxide synthase in both endothelial and airway epithelial cells resulted in diminished airway inflammation [22]. Under normal conditions of NO/cGMP signaling, PDE4, with a high Km for cAMP, is thought to degrade cAMP because PDE3 with a lower Km for cAMP is inhibited by endogenous cGMP and thus can increase cAMP [23]. PDE3-induced vasorelaxation is potentiated when NO/cGMP is suppressed as PDE3 inhibition increases both cAMP and cGMP, in which cGMP inhibits cAMP degradation. PDE4 inhibition only increases cAMP and thus is unaffected by NO/cGMP suppression [23]. PDE3 seems to be more responsible for cAMP degradation at low intracellular cAMP concentrations, whereas PDE4 is more important for control of cAMP at higher concentrations [24]. This suggests a beneficial effect of NO in allergic airway inflammation and urges caution in the use of NOS inhibitors [22]. Since the first PDE3 inhibition papers in the 1990s, 11 PDE families have been identified, and presently at least four isoforms of PDE4 are known [25]. Also, the idea of signalosomes has been postulated and partly verified [26].

### 4. Modulation of structural cells and immune cells by PDE3 and 4 inhibition

Several structural cells express PDE3. Inhibition of PDEs has a number of consequences in the pathophysiology of asthma.
4.1. Smooth muscle cells and cardiomyocytes

Cardiac muscle tissue and smooth muscles are not under conscious control. The role of PDE3 in cardiac muscle and in vascular and bronchial smooth muscle slightly differs due to regulation by different modulators and inhibitors [19]. Vascular SMC and airway SMC are widely comparable [27]. Reducing cAMP by PDE3 modulates contraction; PDE3 inhibition (PDE3i) leads to relaxation of vascular and airway SMC which results in vasodilation and bronchodilation due to the elevated levels of cAMP. NO activates soluble- and membrane-bound guanylate cyclases, which synthesize cyclic guanylate monophosphate (cGMP), which subsequently can serve as a competitive inhibitor of PDE3 as well as activator of cGMP protein kinases [16]. The downstream effects of NO are limited, in part, by phosphodiesterase (PDE)-induced degradation of cGMP [28].

The primary mechanism behind the PDE3 regulation of myocardial physiology relates to its control of cAMP levels; inhibition of myocardial PDE3, especially PDE3A, leads to decreased cAMP breakdown, resulting in increased cAMP which mediates positive inotropic effects and increases in myocardial contractility [29]. Although PDE3 inhibitors increase myocardial contractility and vasodilation in heart failure patients [29], prolonged use of the PDE3 inhibitor milrinone in these patients increased mortality was observed, most likely due to arrhythmias and cardiac arrest [30]. Presently, milrinone has an approval for short term treatment of untreatable exacerbations of heart failure and as a chemical “bridge to transplant” [31]. The work of Chen Yan and her colleagues suggests that the untoward effects of chronic administration of relatively high dosis of milrinone may possibly be related to long term effects of cAMP on pathological remodeling and progression of heart failure [32], via upregulation of inducible cAMP early repressor (ICER) and subsequent increases in cardiomyocyte apoptosis [33]. According to this hypothesis, PDE3 inhibitors increase cAMP, leading to increased expression of ICER, which blocks transcription of PDE3. This cascade of events induced a pathological “feedback loop,” with downregulation or inhibition of PDE3 leading to increased cAMP/PKA signaling, upregulation of ICER, continued downregulation of PDE3, and enhanced apoptosis in cardiomyocytes [33].

In smooth muscle cells, increased cGMP levels induce vasorelaxation. Due to effects of PDEs on hydrolysis of cGMP, PDE inhibitors play a major role in the fine-tuned regulation of this function. In addition to PDEs, NO plays an important role in vasorelaxation, perhaps, in part, by its activation of cytosolic guanylate cyclases, leading to increased production of cGMP, and subsequent inhibition of PDE3. The PDE3 inhibitor, cilostazol (Pletal), is widely used to treat intermittent claudication (IC), a lower-extremity peripheral arterial disease characterized by exercise-/ischemia-induced leg pain. It is thought that cilostazol increases walking distance and alleviates IC symptoms by cAMP-mediated vasodilation and inhibition of both platelet activation and vascular wall inflammation [34].

Asthma can present itself with varying levels of severity, and a particular subgroup of patients, labeled as “severe asthmatics” is characterized by the persistence of symptoms despite therapy with corticosteroids [2, 35]. Examination of bronchial airways from patients with severe asthma shows a greater amount of ASM (Airway Smooth Muscle) cell mass and of subepithelial fibrosis compared to non-severe asthmatics [36, 37]. In ex-vivo studies, ASM cells from severe asthmatics demonstrated increased cell growth and proliferation [38] and an increase in proliferating cell nuclear antigen, a marker of proliferation [39]. Cultured ASM cells from mild-to-moderate
asthmatics also proliferated faster than ASM cells from normal subjects [40]. Bhavasar et al. have previously demonstrated corticosteroid insensitivity in blood monocytes and alveolar macrophages from patients with severe asthma compared to those with non-severe asthma [41, 42]. Another feature of steroid insensitivity could be the ongoing ASM cell growth because the enhanced proliferation of ASM cells from patients with mild asthma is resistant to dexamethasone [43]. Given this perspective, it is of interest that studies with VSMC from PDE3A and PDE3B KO mice indicated that the absence of PDE3A, not PDE3B, diminished VSMC proliferation and indicated a G0/G1 cell cycle arrest [44]. PDE3 inhibition might reduce ASM proliferation in asthmatics.

4.2. Endothelial cells

Endothelial cells play an important role in the pathophysiology of asthma. Due to the expression of adhesion molecules, they enable cells to extravasate from the bloodstream into the inflamed tissue. Endothelial cells also possess a barrier function to prevent leakage of blood fluid in the tissue. Endothelial cells express PDE3 and 4, and inhibition of PDE3 and 4 of endothelial cells inhibited eosinophil and neutrophil adherence to monolayers of endothelial cells [45, 46]. PDE3 and 4 synergistically enhance the inhibition of VCAM1 expression and eosinophil adhesion to activated-human lung microvascular endothelial cells [45]. Inhibition of PDE3 leads to increases in cAMP which improves endothelial barrier functions and supports cell-cell junctions [47]. BW245c, a DP receptor antagonist, increases cAMP, and enhanced endothelial barrier function in a cAMP-dependent matter via the DP receptor, a G protein coupled receptor [48, 49]. Hyperpermeability of pulmonary endothelial monolayers, evoked by thrombin or Escherichia coli hemolysin, can be blocked by the simultaneous activation of adenylyl cyclase and inhibition of PDEs, especially PDE3 and PDE4 [50]. Sphingosine-1-phosphate (S1P1) induces endothelial cytoskeletal rearrangement and barrier enhancement by S1P1 receptor, PI3 kinase, Tiam1/Rac1, and alpha-actinin dependent mechanisms as well [51]. In vivo studies with asthma models indicate that compounds such as BW245c, sphingosine 1-phosphate receptor agonist (FTY720), and prostacyclin-2 analog (iloprost) impair Dendritic Cell (DC) migration [52–54]. This can be explained by a direct effect of these compounds on improving endothelial cell barrier function via elevated levels of cAMP [48, 55], which might affect DC migration from tissue to the draining lymph nodes.

PDE inhibition, a therapeutic approach to increase cAMP levels, was beneficial in treating capillary leakage and edema in a rat model of systemic inflammation induced by LPS [56]. Moreover, PDE3 inhibition was compared to dobutamine treatment (β-adrenoreceptor compounds); the former showed inotropic, lusitropic, and vasodilating properties which were not seen in patients treated with dobutamine [57, 58]. In bypass surgery patients, reduced inflammatory responses were observed during PDE3 inhibition compared to placebo treatment [59]. Furthermore, reduced TNF-α-levels, a cytokine which is increased in sepsis, were observed during PDE3 inhibition by enoximone compared to dobutamine-treated septic patients [58].

Hydrogen peroxide (H$_2$O$_2$), derived from neutrophils and other cells, supposedly is important in the development of vascular injury and thus of pulmonary edema. In a porcine pulmonary artery endothelial cell monolayer model, H2O2 increased hydraulic conductivity while selectivity was decreased. It is known that certain inhibitors of PDE isozymes 2, 3, and 4 could block H$_2$O$_2$-induced endothelial permeability [60]. The data suggest that adenylate cyclase activation/PDE inhibition is a powerful approach to block H$_2$O$_2$-induced increase in...
endothelial permeability. This concept appears especially valuable when endothelial PDE isoenzyme patterns and PDE inhibitor profiles are matched optimally [61].

4.3. Epithelial cells, pneumocyte type I and type II

Human epithelial cells express PDE3 [62]. NO and cAMP both modulate membrane water permeability via aquaporin5 expression in pneumocyte type I [63, 64]. Experimental lung edema can be attenuated by selective PDE3 and PDE4 inhibitors [50, 65–67]. In experimental pulmonary edema, PDE3 inhibition reduces the numbers of inflammatory cells in BAL [66]. In alveolar epithelial cells, LPS-induced biosynthesis of proinflammatory cytokines is regulated by cAMP and tightly controlled by PDEs, and can be reduced by PDE inhibitors [68].

Inhibition of PDE3 and elevation of cAMP improve epithelial and endothelial barrier function and reduce SMC proliferation, which are interesting therapeutic targets in the future for asthma.

5. Immune cells

Mechanisms for regulation of PDE3 activity in immune cells, including dendritic cells, monocytes, B-cells, NK cells γδT cells, αβT-cells, T-cells, macrophages, eosinophils, and neutrophils, all of which express PDE3 isoforms are largely unknown (immgen database http://www.immgen.org/databrowser/index.html). Theophylline is a nonspecific PDE inhibitor [69]. In asthmatic patients, the inflamed airway mucosa, characterized by the presence of eosinophils, IgE positive mast cells, T-cells and dendritic cells, exhibits dysregulated barrier immunity [70]. These various inflammatory cells each have their own position in the asthma cascade. PDE3 and PDE4 are the major isoenzymes regulating IgE-stimulated mediator release from rat peritoneal mast cells [71]. Alveolar macrophage activation can be inhibited by PDE3/PDE4 inhibitors [72]. DC cultures were treated with a PDE4 inhibitor and with combined inhibition of PDE3 and 4; the latter resulted in a two times stronger reduction in LPS-induced TNFα release in DC cultures [73]. In vitro inhibition of PDE4 in DCs resulted in reduced development of Th1 cells as measured in reduced capacity to produce IL-12p70 and TNFα upon LPS or CD40L stimulation [74]. Peripheral blood monocytes from atopic dermatitis patients and healthy controls show inhibition of LPS-induced TNFα release during treatment with PDE4 inhibitors [75].

Inhibition of PDE3 and PDE4 prevents immunogen-stimulated IL-2 release from CD4 and CD8 human T-cells. Human T-cells and B-cell express PDE3 [73, 76–78]. Knock down strategies or inhibitors of PDE4B or D inhibit IL-4, IL-5, and IFNγ expression or production [79–81]. Peripheral blood mononuclear cells from atopic dermatitis patients and healthy controls show inhibition of PMA-induced proliferation due to the treatment with PDE4 inhibitors. cAMP was found to inhibit T-cell proliferation and differentiation which was linked to IL-2 [82, 83]. IL-2 activation of CD25+ T cells (Treg cells) led to a drastic upregulation in AC activity and to cAMP accumulation; an opposite significant decrease in AC activity was seen in CD25–T cells [83]. The PDE activity remained unchanged in both cell subpopulations, suggesting that the mechanism of cAMP accumulation in stimulated Treg involves AC7 activation [83].
Cyclic AMP is a pleiotropic regulator of cell growth and function. In T-cells, cAMP suppresses TCR-triggered proliferation and cytokine production. cAMP is also a selective modulator of the actions of the proinflammatory transcription factor NF-κB. NF-κB plays a crucial role in switching on the gene expression of inflammatory and immune mediators and is therefore an important target for therapy [84]. cAMP is an important negative regulator of T cell activation, and increased levels of cAMP are associated with T cell hyporesponsiveness in vitro [85]. Stimulation of mouse CD4 T-cells by immature allogeneic DC combined with a PDE3 inhibitor resulted in functional Foxp3+ T-cells that delayed allograft rejection [86]. Moreover, PDE3 inhibition results in functional human Foxp3+ CD4+ T-cells which are driven by allogeneic APCs. The mechanism for these responses seems to be related to demethylation of FoxP3 gene [86].

Treatment with S-Petasin, an inhibitor of PDE3 and 4, reduced eosinophilic airway inflammation in an OVA model for asthma [87]. Although eosinophils do not express PDE3, reduced inflammation might be an indirect consequence of elevated levels of cAMP in endothelial cells that enhance endothelial barrier function and lowered the expression of adhesion molecules [45, 47]. PDE3 inhibitors sustained increased levels of cAMP in mast cells which are inhibitory to both basophils and human lung mast cells function [88]. Rat peritoneal mast cells showed reduced IgE-stimulated mediator release when treated with PDE3 inhibitors [71]. The conductive players in asthma, including T-cells and DC, and the central effector cells in asthma, including eosinophils, mast cells, basophils and neutrophils, can be targeted directly or indirectly with PDE3 inhibitors.

Recently, more and more interest is seen for the “old” theophylline which is a broad PDE inhibitor [69]. Theophylline [69] is a drug which targets PDE4 and, at high doses, also PDE3. However, it is a relatively weak bronchodilator at therapeutic concentrations. In patients, it is beneficial; and addition of theophylline can improve asthma control to a greater extent than beta2-agonist in patients with severe asthma [89]. Furthermore, in asthma patients poorly controlled by steroids, low dose theophylline added to inhaled corticosteroids improves asthma control [69]. The proposed mechanisms of action of theophylline include nonselective inhibition of PDE, antagonism of Adenosine receptors, inhibition of nuclear translocation of NF-κB, improved histone diacetylase 2, improved IL-10 secretion, induction of apoptosis of inflammatory cells (neutrophils and eosinophils) [90, 91], and inhibition of T-cell proliferation [85]. These features are of significant importance for severe asthma with poor steroid control, in which neutrophils are found and these patients were difficult to treat [4]. Theophylline exerted proapoptotic effects on monocyte-derived dendritic cells (DCs) and impaired DCs differentiation [90, 91].

6. PDE3 and 4 inhibition in the context of asthma

There is little literature available regarding enoximone in the context of airway disease. Bethke et al. showed that enoximone has inhibitory capacity on PDE3 and PDE4 [92]. Fujimura et al. researched cilostazol as a PDE3 inhibitor in asthma, showing its beneficial effect on bronchial hyper-responsiveness in elder asthmatics [93]. PDE4 inhibitors have been described in preclinical and clinical settings in the context of lung diseases like asthma and COPD [94, 95]. The PDE4 inhibitor rolflumilast inhibits TGFβ-induced connective tissue growth factor (CTGF), collagen I and fibronectin in airway smooth muscle (ASM) cells of bronchial tissue rings [96].
Roflumilast is approved as part of the treatment regimen for Chronic Obstructive Pulmonary Disease (COPD) [97]. PDE3 inhibitors, including cilastozol, milrinone, and mixed PDE3/4 inhibitor enoximone, have mainly been used in the context of heart failure. Literature provides several cases with adverse effect and fatal outcome in the use of high dose PDE inhibitors for the chronic treatment of severe heart failure. A reason for this unfavorable outcome might have been that enoximone in heart failure was given in exceedingly high doses up to 2400 mg daily (31 mg/kg/dd) [98–100]; doses which were found to be extremely likely to cause severe side effects and a high mortality rate: after 6 months of treatment, at least half of the patients had died. Thus, the early research in pulmonary use has been abandoned and, since the late 90s, the sparse research into use of PDE3-inhibitors for pulmonary purposes has not led to the use of any of these drugs in the treatment of asthma. The first paper addressing actual clinical cases, in which enoximone treatment was given successfully in status asthmaticus and near fatal asthma was Beute [3], inspired by the resemblance between vascular and bronchial smooth muscle cell relaxation. In this paper, the doses used were considerably lower (1.15 mg/kg single dose) and the duration of administration was substantially shorter than in heart failure. Here, enoximone proved to be beneficial without side effects.

Enoximone is known as a PDE3 inhibitor that increases levels of cAMP as well as cGMP; however, in those cells where both compounds are present, cGMP will act as a competitive inhibitor on the breakdown of cAMP, thereby sustaining elevated levels of cAMP. cGMP can also be generated by nitric oxide (NO)-induced stimulation of guanylyl cyclase (both abundantly present in smooth muscle cells), again impairing the breakdown of cAMP. The IC50 values of enoximone for PDE3 and PDE4 are 5.9 and 21.1 μM. The affinity of PDE3 for cAMP is 20 times higher than that of PDE4 [101].

These mechanisms probably allow for the favorable outcome of the relatively small doses of enoximone in Beute [3] and suggest an effect that exceeds its half-life.

Smooth muscle relaxation is more pronounced after administration of selective PDE3 inhibitors compared with PDE4 inhibitors. PDE3 inhibition leads to the enhancement of relaxation evoked by β2-receptor stimulation. Furthermore, simultaneous administration of siguazodan (PDE3 inhibitor) and rolipram (PDE4 inhibitor) enhances this relaxation, [102].

In Figure 1, both PDE3 and 4 are important in tailoring cyclic adenosine monophosphate signaling. PDE3/4 inhibitor increases intracellular cyclic adenosine monophosphate levels and has anti-inflammatory effects. Activation of a G-protein-coupled receptor (GPCR) activates adenylyl cyclase (AC) resulting in the induction of cAMP with the consequence of phosphokinase A (PKA) activation. Effect of PDE3/4 inhibition causes bronchodilation and improves endothelial and epithelial barrier function.

PDE4 is also present alongside the PDE3 isoenzyme in airway smooth muscle; the PDE3 isoenzyme is considered to predominate in airway smooth muscle, and inhibition of this enzyme leads to airway smooth muscle relaxation [103]. Moreover, PDE3 isoenzyme A is located in the cell membrane [25] and presumably easy to target, and could be involved in the rapid effects of therapy (minutes or earlier) seen during the intravenous emergency treatment in the studies of Beute [3].

Bringing to mind once again that all the cells and mechanisms mentioned in this chapter are regulated/influenced by either PDE3, PDE4, or both, and that all these cells and mechanisms
are involved in the development, maintenance, or aggravation of asthma, there is a strong case for the assumption that enoximone may have a large impact on the acute treatment of severe asthma, on various separate levels. Additional safety studies will also be required.

As discussed above, further research in PDEs appears to be advisable in order to investigate their true potential.

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