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Neprilysin inhibitors as a new approach in the treatment of right heart failure in the course of chronic obstructive pulmonary disease

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Abstract

The aim of this paper was to review scientific evidence on the possible use of the combined angiotensin II receptor antagonist and neprilysin inhibitors (ARNI) in patients with right heart failure (RHF) in the course of chronic obstructive pulmonary disease (COPD). It has been proven that a lack of neprilysin or its reduced expression in hypoxia leads to exacerbation of pulmonary arterial remodelling (PAR) or pulmonary hypertension (PH) in the mechanism related to the platelet-derived growth factor (PDGF) resulting in the proliferation and migration of pulmonary artery smooth muscle cells and endothelial-to-mesenchymal transition. Such action in the course of COPD can lead to RHF, which would signify noxious effect of this group of drugs. However, the inhibition of neprilysin also hinders natriuretic peptide metabolism. The representative of this group — brain natriuretic peptide (BNP) — acts as a vasodilator and also exerts an antiproliferative activity through the cGMP-dependent protein kinase G pathway. Additionally, it causes bronchodilation by inducing the release of acetylcholine from bronchial epithelial cells. This suggests that natriuretic peptides may appear to be a potential treatment agent in patients with cardiac complications and COPD. Their effects associated with the immunosuppression capacity by reducing the release of inflammatory mediators — IL-6, IL-1β, and TNF-α can bring benefits to patients with acute lung injury caused by pulmonary inflammation during COPD exacerbations. Considering the potentially positive effect of natriuretic peptides in this group of patients, further research is required in this area, which can provide strong scientific data demonstrating the need for introducing ARNI drugs to the treatment of patients with COPD.

Key words: sacubitril, neprilysin, natriuretic peptides, right heart failure, chronic obstructive pulmonary disease

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Introduction

Chronic obstructive pulmonary disease (COPD) is an incurable, progressive systemic disease affecting more and more people in the world. Epidemiological data indicates that in Poland COPD is diagnosed in about 10% of Poles on the basis of spirometry values greater than or equal to 2 according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines [1]. Some reports claim that by 2020, COPD will become the third leading cause of death in the world after cancer and accidents [2].

This disease is characterised by permanent limitation of airflow through the airways and their increased inflammatory reaction in response to tobacco smoke, biomass-containing fuels, diesel exhausts, and infections. The main risk factor for the development of COPD is tobacco smoke. This factor has been estimated to account for up to 80% of cases [1]. COPD induces and coexists with numerous diseases.

COPD is not only a lung disease but a multi-organ and systemic disease as well. Chronic respiratory failure affects physical activity, which in turn may lead to metabolic problems [3] and

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an increased risk of embolism [4]. The progression of COPD results in a significant number of complications.

One of the most serious and difficult to treat consequences of the disease progression is the right heart failure (RHF). This process is affected by pulmonary hypertension (PH), which arises from chronic hypoxia, destructive effects of tobacco smoke and inflammation; these factors induce the shrinkage of small pulmonary arteries and their remodelling. The persistence of chronic inflammation over time leads to morphological changes in the pulmonary vessels and an increase in vascular resistance, the consequence of which is RHF. Capillary destruction is likely to increase this process in the course of emphysema, which is also one of the COPD complications [1].

Therapeutic possibilities in COPD

COPD with numerous comorbidities requires multidirectional treatment and multidisciplinary care in order to optimally reduce symptoms and the risk of future exacerbations. Therapeutic management should therefore be thought through, should be consistent, understandable for the patient and should include all comorbidities as well as the likelihood of their development and progression [1]. However, polypragmasia must be considered while establishing the treatment. Apart from administration of inhaled drugs, which are the main agents of the therapy, the patient should be motivated to quit smoking [1]. Physical activity plays a significant role in the therapy, and improvement in the patient's condition can eliminate disease symptoms or reduce their severity [5]. Properly tailored treatment will not only improve respiratory efficiency but will also have a positive effect on the functioning of the entire patient's body, including cognitive functions. Patients achieve beneficial results also through respiratory rehabilitation [6]. Moreover, adequate nutrition exerts an impact on the efficacy of therapy and achievement of positive results [1]. Equally important and often neglected by practicing physicians is patient's education; knowledge of the disease and awareness of its consequences improve disease control and increase the chance for satisfactory results of therapy. Prophylaxis and recommended vaccination against influenza and pneumococcal infection are also important factors in COPD [1].

ARNI as a novel therapy for heart failure in patients with COPD

One of the more recent and more promising therapies for heart failure is the administration of the combined angiotensin receptor blockers (ARBs) — valsartan, with the neprilysin (NEP) inhibitor — sacubitril [7]. Sacubitril by inhibiting neprilysin prevents the disintegration of natriuretic peptides, mainly brain natriuretic peptide (BNP) and atrial natriuretic peptide (ANP). NEP inhibition leads to an increase in the bioavailability of natriuretic peptides, which have a beneficial effect on the cardiovascular system in heart failure — they increase natriuresis and diuresis and act as vasodilators. Valsartan and sacubitril exert an advantageous action in the prevention of heart remodelling [7]. The use of the above-mentioned combined substances may have a very beneficial pleiotropic effect on the circulatory system of patients, arising from the multidirectional action of this drug. Valsartan has been shown to suppress myocardial remodelling by inhibiting the guanine nucleotide-binding protein family, while sacubitril prevents myocardial cell death by inhibiting phosphatase and tensin homologue (PTEN) [7]. The additive effect of these substances causes the reduction in the left ventricular extracellular matrix remodelling (LVEMR). [7] Increasing the concentration of natriuretic peptides leads to a drop in arterial blood pressure due to the inhibition of renin secretion and subsequent blocking of the renin-angiotensin-aldosterone system.

The use of this new group of drugs in RHF in the course of COPD has been evidenced by scarce scientific data, which indicates the need for further research. Only a few studies combine neprilysin inhibitors with pulmonary hypertension and RHF. These works have shown efficacy of using neprilysin inhibitors in reducing pulmonary hypertension (PH) and impeding RHF progression, which was associated with the inhibition of ANP metabolism [8–10]. However, the studies were carried out in the 1990s only on animal models. This encouraged the authors of the present study to perform a detailed analysis of the role of natriuretic peptides and neprilysin in PH, COPD and RHF. The purpose of the analysis was to obtain the information on potential benefits and side effects of using neprilysin inhibitors or natriuretic peptides in the above-mentioned diseases.

It is worth mentioning that only after conducting the PARADIGM-HF clinical trial, a new group of drugs, the angiotensin II receptor blocker

neprilysin inhibitor (ARNI), was introduced to the recommendations for the treatment of heart failure with reduced ejection fraction (HfrEF) according to the American College of Cardiology (ACC), the American Heart Association (AHA), the Heart Failure Society of America (HFSA), and the European Society of Cardiology (ESC) [11]. Sacubitril alone has become the object of interest for many researchers and the following extensive research studies have been undertaken: PARAMOUNT and PARAGON trials on the application of ARNI in heart failure with preserved ejection fraction (HFpEF); LIFE, PIONIER-HF in HFrEF; or PARADISE-MI in the secondary prevention of infarction [11].

Neprilysin as an intra-membrane metalloproteinase

The therapeutic effect of sacubitril is associated with an increase in the concentration of natriuretic peptides. To achieve this effect, it is necessary to inhibit neprilysin (neutral endopeptidase, NEP, CD10), which is an intra-membrane metalloproteinase located on chromosome 3q 25.1-q25.2 [12] involved in the metabolism of many proteins, among others, substance P, bradykinin, cholecystokinin [13, 14] or endothelin. This protein also demonstrates a wide expression in the body — it has been identified, among others, in the lungs [15], prostate gland [16], kidneys [17], and brain [18]. In the light of the above scientific data, we have put forward the hypothesis that the inhibition of such a thorough enzyme will not remain completely neutral for cardiac performance in patients with COPD.

Neprilysin-dependent vascular remodelling

Decreased expression of NEP or lack of this enzyme have been associated with pulmonary vascular remodelling in response to chronic hypoxia [19]. In addition, NEP expression was reduced in the lungs of COPD patients [20], which may possibly exacerbate pulmonary vascular remodeling in COPD [20]. COPD often leads to PH [21, 22], which is connected with chronic hypoxia, noxious effects of tobacco smoke, free radicals and inflammatory mediators, such as IL-6 [21–24]. In the course of PH, pulmonary vascular remodelling arises through proliferation and migration of pulmonary artery smooth muscle cells (PASMCs) [19, 20, 23, 25–27] and endothelial-to- mesenchymal transition (EndoMT) [28]. Ultimately, this hypertension can lead to RHF [22]. Severe PH is a relatively rare complication of COPD [21, 29], however, it is associated with a worse prognosis and an increased risk of death [21].

The molecular basis of these mechanisms has not been fully understood. The platelet-derived growth factor (PDGF) and receptors for PDGF (PDGFR) play an important role in the pulmonary vascular remodelling and PH [27, 30]. Stimulation of PDGFR and elevated PDGF concentration lead to increased proliferation and migration of PASMCs [25], and induce EndoMT [28]. Interestingly, the decrease in the amount or total absence of NEP in PASMCs was associated with an increased amount of PDGF and PDGFR [25, 28]. The reason for this is the excessive activation of the cytoplasmic Src kinase and phosphate and tensin homologue (PTEN) phosphorylation due to the reduced amount of neprilysin, which finally leads to PDGFR activation [25, 31]. Moreover, in the absence of NEP, the concentration of its substrates, such as endothelin 1 (ET-1) and fibroblast growth factor (FGF-2) significantly elevating the PDGF-induced migration and proliferation [25], is increased. Additionally, ET-1 as a strong vasoconstrictor plays a significant role in PH [32]. In response to hypoxia, a decreased NEP concentration [28] and increased level of PDGF as well as transforming growth factor β1 (TGF-β1), with a positive regulation observed between them, were stated in pulmonary arterial endothelial cells (PAECs). This state induced EndoMT [28]. Despite numerous scientific data demonstrating the negative impact of TGF-β1 in hypertension [33, 34] and pulmonary vascular remodelling [35], there is scientific evidence linking the excessive expression of TGF-β type II receptor with antiproliferative activity in the pulmonary vessels [36]. This can play a protective role in the development of pulmonary hypertension occurring in the final stage of severe COPD. Neprilysin in an animal model inhibited hypoxia-induced EndoMT and decreased PDGF and TGF-β1 levels in PAECs; and the addition of soluble small interfering siRNA to neprilysin (si-NEP) under normoxic conditions induced EndoMT [28]. It should be remembered that PAR is an extremely complex process in which a whole range of other growth factors and inflammatory agents are included, however without PDGF or NEP involvement [30].

The experimental model showed that the loss of NEP did not result in considerable changes in the respiratory and circulatory system under normoxic conditions [19]. Neuroendocrine cell (NEC) hyperplasia was the only change in this study [19]. This hyperplasia was also obtained while admin-

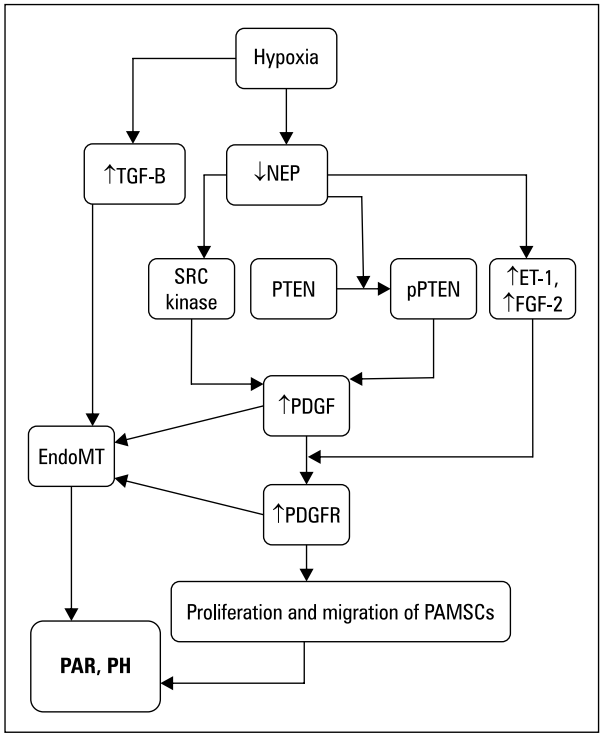

Figure 1. Molecular mechanisms of pulmonary artery remodelling and pulmonary hypertension

istering NEP inhibitors [37]. In some works, the pulmonary neuroendocrine cell hyperplasia has been associated with PH [19, 38, 39]. NECs are responsible for the secretion of such substances as ET-1, serotonin (5-HT) or substance P, whose concentration was significantly increased in NEC hyperplasia in the absence of NEP [19]. Other papers indicated the potentially positive effect of neprilysin inhibitors in PH and PAR [9, 10], and one study [19] showed a slight difference in right ventricle (RV) hypertrophy in hypoxia: NEP-free mice had a smaller RV hypertrophy than mice with NEP.

The cited studies were carried out on small test groups (4 mice) [19], therefore it is difficult to draw clear conclusions on the effect of neprilysin in COPD.

The role and expression of NEP in other diseases

In small-cell lung cancer, a decreased expression of neprilysin occurs which hydrolyses, among others, bombesin-like peptides [40] that can function as autocrine growth factors and serve as a suppressor in this tumour [41]. This suggests that the inhibition of this enzyme may cause a faster progression of this cancer. It is also worth mentioning that cigarette smoking is one of the reasons for decreasing the activity of neprilysin in the lungs through the free radical-dependent mechanism [40]. Cigarette smoking increases the risk of developing COPD and small-cell lung cancer, while in the prostate gland, the NEP expression is positively regulated by androgens. In the advanced stage of prostate cancer, this mechanism is lost, which leads to the reduced expression of this metalloproteinase [42]. Moreover, the decrease in this enzyme was also observed in clear-cell and chromophobe renal-cell carcinoma, which is associated with the loss of the antitumour action of vasoactive intestinal peptide (VIP) [43]. In cervical [44] and breast cancer [45, 46], anti-progressive action of neprilysin was also demonstrated.

The involvement of neprilysin in beta-amyloid hydrolysis is also known [47]. Deposits of beta-amyloid may cause Alzheimer's disease. This shows that the effect of ARNI may influence not only the lungs but also may act in a systemic way, and in the case of patients at high risk of the above-mentioned diseases may have a noxious impact accelerating the development of the disease.

Natriuretic peptides as novel therapeutic possibilities in patients with COPD

Structure of natriuretic peptides

Natriuretic peptides are a group of proteins synthesised and secreted mainly by the heart of mammals [48]. Atrial natriuretic peptide (ANP) is stored in atrial cardiomyocytes in the form of proANP (1-126), which is released into the blood and digested by corin into biologically active ANP (1-28) and inactive ANP (1-98) [49]. The main stimulant of ANP secretion is volume overload of the atria caused mainly by high venous pressure. Other factors causing the increase in ANP secretion are the following: tachycardia, endothelin, vasopressin, catecholamines, hypoxia, increase in osmolarity, thyroid hormones, glucocorticoids, TNFα and other inflammatory cytokines [49, 50]. Brain natriuretic peptide (BNP) is synthesised mainly in cardiac ventricular cardiomyocytes. Due to the action of corin on proBNP (1-108), biologically active BNP (1-32) and inactive NT-proBNP are formed. Unlike ANP, biologically active BNP is released into the bloodstream immediately after synthesis, although small amounts can be stored in the atrial granules and ventricular tissue [49]. In a healthy human, the concentration of this peptide is very small, 0.5–30 pg/ml [51], howev-

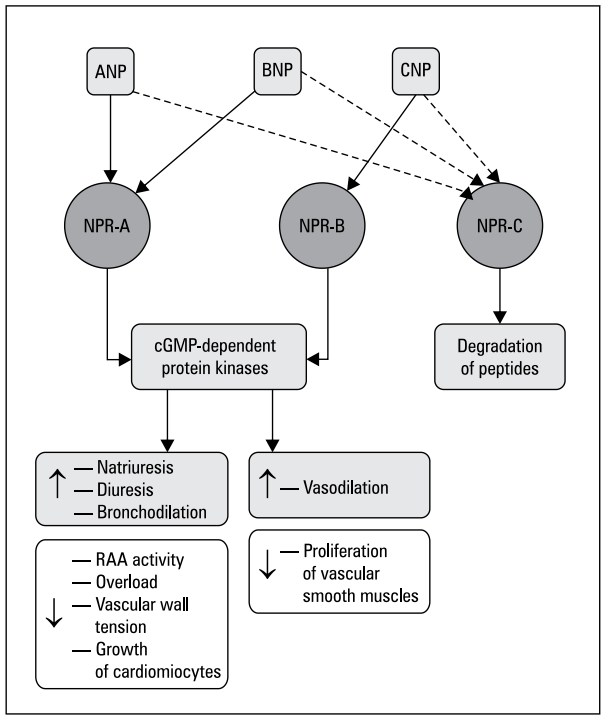

Figure 2. Role of the natriuretic peptides

er, in patients with chronic heart failure, it can be significantly increased even by > 500 pg/ml [52, 53]. C-type natriuretic peptide (CNP) occurs in two biologically active forms, CNP-53 and CNP-22, and is secreted mainly by the vascular endothelium. The stimulation of CNP secretion is primarily caused by the transforming growth factor β (TGF-β), and also by tumour necrotic factor (TNF), interleukin-1 (Il-1), and fibroblast growth factor-2 (FGF-2) [52].

Mechanism of action and elimination of natriuretic peptides

So far, three natriuretic peptide receptors, NPR-A, NPR-B, NPR-C, have been known. The first two belong to the transmembrane proteins from the family of receptors coupled to guanylate cyclase [48]. The biological activity of ANP and BNP is based on the interaction with the transmembrane NPR-A receptor, which leads to the conversion of guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP). This causes the activation of cGMP-dependent protein kinases, which results in the physiological effect of natriuretic peptides [48, 54, 55]. The ANP/NPR-A system is responsible for the decrease in heart overload by reducing vascular wall tension, stimulating natriuresis and diuresis and inhibiting the renin-angiotensin-aldosterone pathway and the growth of cardiomyocytes [54]. CNP affects NPR-B, and similarly to NPR-A, its activation causes the increase in cGMP. The CNP/NPR-B system is responsible for vasodilation and antiproliferative effect on vascular smooth muscles as well as the regulation of the growth of long bones and vertebral bodies [56].

The removal of natriuretic peptides takes place through binding to NPR-C followed by their enzymatic lysosomal degradation, neprilysin action, and renal excretion [48].

Effect of natriuretic peptides on the respiratory system in healthy people and patients with COPD

Natriuretic peptides are produced in a large amount and secreted by the bronchial epithelium, pulmonary alveolar epithelium, and Clara cells [57]. The degradation of these peptides also occurs to a large extent in the lungs [58].

Vasodilation and antiproliferative activity

Numerous studies have shown that the concentration of ANP and BNP significantly increases in PH [58, 59], suggesting their crucial role in the regulation of the pulmonary circulation. In COPD patients, pulmonary hypertension is associated with vasoconstriction caused by chronic hypoxia [60]. In patients suffering from chronic obstructive pulmonary disease, BNP levels are often elevated especially with concomitant left hair failure (LHF), PAR and right heart remodelling [61] — these are diseases that often accompany COPD. In experiments on mice with pulmonary hypertension induced by chronic hypoxia, after administration of BNP (1.4 μg/min), a decrease in systolic pressure in the right ventricle and its mass was observed as compared to the control group. This effect was associated with reduced pulmonary vascular resistance induced by BNP [62]. Another study demonstrated the antiproliferative effect of BNP on PASMCs. It has been proven that BNP inhibits the proliferation and migration of PASMCs induced by angiotensin II. These effects are potentially mediated by decreased calcium influx, reduced ROS production by Nox1 and mitochondria, and down-regulation of MAPK and Akt signal transduction, through the cGMP/PKG pathway [63]. In COPD patients with secondary hypertension induced by hypoxia following a synthetic human ANP infusion, a decrease in systemic and pulmonary arterial pressure was observed, resulting in an increase in cardiac

output (CO) with the unchanged heart rate and preload. In addition, aldosterone levels decreased in these patients [60]. In another study, ANP and BNP were found to cause dose-dependent vasodilation of pulmonary vessels in patients with cor pulmonale [64].

Bronchodilation

For many years, scientific studies have been focused on the impact of natriuretic peptides on bronchial obstruction. The research performed on animals has proven their bronchodilatory activity. Unfortunately, there are few studies confirming the effect of natriuretic peptides on human bronchospasm. However, it has been evidenced that administration of nesiritide (recombinant human BNP) resulted in significant bronchodilation in asthmatic patients suggesting that this substance has a beneficial effect on the respiratory tract and may be used as an additional treatment in patients with acute exacerbations of asthma resistant to standard treatment [65]. Unfortunately, there are no such studies concerning the effect of nesiritide on COPD patients. The bronchodilator activity of BNP is most likely associated with the activation of the NPR-A receptor located on the respiratory endothelium, which in consequence causes the alveolar release of very low concentrations of acetylcholine from the bronchial epithelial cells. The picomolar concentrations of acetylcholine activate the postsynaptic M2 muscarinic receptors located on airway smooth muscle (ASM) cells. The activated M2 receptor modulates the inducible nitric oxide synthase (iNOS) gene and protein expression, causing an increase in nitric oxide (NO) levels in ASM and activation of NO/cGMP signalling, which results in bronchial relaxation [66]. There are some studies on the use of inhaled natriuretic peptides. It has been proven that ANP inhaled by asthma patients causes bronchodilation, however, this effect lasts for a short time, as it is rapidly metabolised by neprilysin. The addition of a neutral endopeptidase inhibitor to ANP resulted in prolongation of bronchodilation time, however, it was still shorter compared to salbutamol [67]. On the other hand, there are some studies which demonstrate the beneficial effect of neutral endopeptidase on the respiratory tract. Neprilysin plays a significant role in the degradation of neuropeptides in the airways and therefore participates in the regulation of bronchial tonus in animals and humans. NEP is responsible for inactivating almost 90% of strong tachykinins that selectively bind to substance P and are responsible for bronchoconstriction [68]. Therefore, there are contradictory results as regards the therapeutic application of endopeptidase inhibitors to enhance and prolong the action of natriuretic peptides. The NPR agonists are an alternative. PL-3994 — a strong selective NPR agonist, resistant to NEP action, causes relaxation of smooth muscles of guinea pig and human respiratory tract. It also exerts a longer effect compared to ANP, which suggests its potential benefits in patients with asthma [69] and other obstructive pulmonary diseases. The above studies confirm that natriuretic peptides may serve as additional treatment of patients suffering from asthma or COPD with accompanying cardiac complications. BNP combined with existing bronchodilators might be an additional drug in the therapy of patients refractory to standard treatment. However, further research is needed in this area.

Immunosuppressive activity

Natriuretic peptides also affect the immune system. The ANP/GC-A/cGMP-dependent signalling pathway has been found to act suppressively on the proinflammatory transcription factors, nuclear factor-kappaB (NF-κB) and activating protein-1 (AP-1), in cells activated by bacterial lipopolysaccharide (LPS), thus reducing the release of inflammatory mediators, i.e. IL-1β, IL-6 or TNF-α [54]. Moreover, ANP has been shown to attenuate the induction of E-selectin by LPS in the *in vitro* study and weakens the penetration of inflammatory cells in acute lung injury. This suggests that ANP may be an additional drug used in the treatment of acute lung injury in the course of pneumonia or acute respiratory distress syndrome (ARDS) [70]. A very interesting finding shows the relationship between CNP and the biofilm produced by *Pseudomonas aeruginosa*. One of the studies demonstrated that eukaryotic CNP reacted with bacterial AmiC resulting in a slight increase in virulence and a decrease in the formation of *Pseudomonas aeruginosa* biofilm. Thus, a thorough analysis of the CNP activity might contribute to the discovery of new therapeutic methods against *Pseudomonas aeruginosa* [57, 71] — a frequent pathogen in patients with cystic fibrosis or COPD.

Oral natriuretic peptides

The synthesis of oral natriuretic peptides is associated with many difficulties. First of all, human NPs have a short half-life (ANP — 3.1 min,

BNP — 22 min [48]) and are rapidly metabolised and removed by the human body. Therefore, nesiritide — recombinant BNP — used in the treatment of chronic heart failure, is applied in continuous intravenous infusion [72]. For a long time, the synthesis of oral natriuretic peptides has been a great challenge for scientists. The digestive enzymes found in the stomach and small intestine as well as the ineffective transport across the intestinal epithelium proved to be powerful barriers [73]. However, for a few years, scientists have been publishing satisfactory effects of the synthesis of oral NPs [74]. It has been proven that the use of short amphiphilic oligomers covalently linked to human BNP (hBNP) prolongs the biological activity of BNP and causes a significant decrease in mean arterial pressure (MAP) in dogs [73]. However, there are no reports on the effect of oral natriuretic peptides on pulmonary pressure — further research is needed in this area.

Conclusions

In the absence of NEP or its decreased expression under hypoxia conditions, PAR and PH increase, which in the course of COPD leads to RHF. This relationship may indicate a possible exacerbation of symptoms when ARNI is used in patients with RHF in the course of COPD. Interestingly, the therapeutic effect of sacubitril on myocardial remodelling and the detrimental effect of reduced expression of NEP in hypoxia based on the severity of PAR depends on the inhibition of PTEN, which may suggest different mechanisms of pulmonary vessels and heart remodelling.

However, the efficacy of ARNI in HFrEF is associated with an increase in the activity of natriuretic peptides and in particular BNP. The vasodilatory and antiproliferative activity of these peptides on pulmonary vessels is known. In addition, they reduce the RV overload. There is evidence that their use could contribute to the potential benefits for COPD patients with accompanying cardiac complications induced by PH. The ambiguity of scientific data on NEP and natriuretic peptides regarding their effect on PAR and PH and their depletion when using NEP inhibitors in RHF encourages researchers to perform further investigation in this field. Increasing the activity of natriuretic peptides may additionally bring benefits to patients with COPD and acute lung injury caused by sudden pulmonary inflammation (exacerbation) due to the immunosuppressive action of these peptides.

Conflict of interest

The authors declare no conflict of interest.

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