Allicin and Pulmonary Arterial Hypertension

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Pulmonary arterial hypertension (PAH) is characterized by pulmonary vascular remodeling. Recent evidence supports that inflammation plays a key role in triggering and maintaining pulmonary vascular remodeling. Recent studies have shown that garlic extract has protective effects in PAH, but the precise role of allicin, a compound derived from garlic, is unknown. Thus, we used allicin to evaluate its effects on inflammation and fibrosis in PAH.

pulmonary arterial hypertension inflammation fibrosis allicin

1. Introduction

Pulmonary arterial hypertension (PAH) is a life-threatening disease characterized by the progressive loss and obstructive remodeling of the pulmonary vascular bed. PAH leads to a progressive elevation in pulmonary arterial pressure (PAP) and pulmonary vascular resistance (PVR), resulting in functional decline and right heart failure [1]. The poor clinical outcome in patients with PAH is determined by the adaptation of right ventricle (RV) function to the increased afterload mediated by the increased contractility with preserved dimensions and stroke volume [2]. Currently, the pathogenesis of PAH remains unclear and involves numerous factors, including endothelial dysfunction, oxidative stress, and the exaggerated infiltration of inflammatory cells, as well as alterations in signaling pathways to maintain cellular identity and functionality as the morphogenetic protein receptor (BMPR2) [3] [4].

In clinical practice, PAH treatments are limited to the production of vasoactive substances such as nitric oxide (NO) and prostacyclin [5]. However, numerous studies have demonstrated that inflammation is associated with the development of experimental and human PAH [6]. The inflammatory process leads to endothelial cell injury and stimulates pulmonary arterial smooth muscle cell (PASMC) proliferation, playing a key role in triggering and maintaining pulmonary vascular remodeling [7][8]. In fact, in experimental models, increased levels of interleukin-1β (IL-1β), IL-6, tumor necrosis factor-α (TNF-α), and nuclear factor-κB (NFκB) were observed [9]. On the other hand, TNF-α inhibition showed a favorable effect on hemodynamics and pulmonary vascular remodeling in experimental PAH [10]. Thus, this evidence supports the role of inflammation in the pathogenesis and progression of PAH. On the other hand, an increased transforming growth factor beta (TGF-β) protein expression can result in anti-apoptotic responses in pulmonary artery endothelial cells (PAEC) and PASMC [11] and can be a hallmark of endothelial cell (EC) dysfunction [12]. In addition, TGF-β can increase the presence of inflammatory markers [13]. Evidence suggests that cytokines and growth factors play a crucial role in hypertrophy and cardiac fibrosis [14]. Understanding the mechanisms promoting this pathologic process is essential to develop new therapeutic options to reduce RV failure and mortality in PAH patients.

Several studies have reported that medicinal plants, nutraceuticals, and phytochemicals exert significant benefits for PAH [15]. These natural compounds have anti-inflammatory, antiproliferative, and anti-vascular remodeling properties [16].

Garlic (*Allium sativum L.*) and its derived products have been widely used for culinary and medicinal purposes in many cultures and civilizations [17]. Garlic extract has protective effects in PAH, but its molecular mechanism is unknown [18]. The compounds present in garlic include protein, carbohydrates, vitamins, and minerals. Garlic is also especially rich in sulfur compounds, such as alliin, ajoenes, sulfides, and disulfides [19].

Allicin is a natural compound produced from the stable precursor S-allyl cysteine-sulfoxide (alliin) by the action of the enzyme alliinase when garlic cloves are crushed or macerated [20]. This compound has shown various beneficial effects, such as antioxidant and anti-inflammatory effects in cardiovascular diseases [21][22].

Allicin exerts its anti-inflammatory effects through several mechanisms. However, in PAH, these mechanisms remain poorly studied or unknown. Thus, the present study aimed to assess if allicin may exert beneficial effects in the progression of experimental PAH. Our results showed that allicin administration had a protective effect in the MCT model through the prevention of RV hypertrophy and increased pulmonary arterial medial wall thickness. In addition, through the modulation of TNF-α, IL-6, IL-1β, TGF-β, and α-SMA, allicin prevented inflammation and fibrosis in lung tissue. The increases in TNF-α, IL-6, and TGF-β in RV tissue were prevented by the allicin treatment. Therefore, these results suggest that allicin protects the RV, which is one of the heart chambers closely related to the severity and progression of PAH.

2. Discussion

In this study, we found that the oral administration of allicin induced a protective effect in MCT-induced PAH by preventing increased pulmonary arterial medial wall thickness and RV hypertrophy. In addition, we found that allicin treatment had anti-inflammatory and anti-fibrotic effects in PAH. In lung tissue, allicin induced low expressions of *Cd68*, TNF-α, IL-1β, IL-6, TGF-β, and α-SMA. In addition, allicin prevented increases in TNF-α, IL-6, and TGF-β in RV tissue, which is one of the heart's four chambers and a determining organ related to the progression and severity of PAH.

Several mechanisms are involved in PAH progression, but the role of inflammation in triggering and maintaining pulmonary vascular remodeling has recently gained relevance [23]. However, therapies are aimed at stimulating vasodilation [24]. In the context of inflammation, several cytokines, such as TNF-α and IL-1β, are increased in PAH patients' serum, which is related to low survival [25][26]. In addition, previous studies have reported that the expressions of TNF-α, IL-1β, and IL-6 are significantly increased in patients and experimental models of MCT-induced PAH [9][12]. In transgenic mice, the overexpression of TNF-α leads to the development of PAH while rats and dogs with MCT-induced PAH have elevated levels of TNF-α in the lung [27][28][29][30]. In addition, mice with an overexpression of IL-6 develop PAH, while knock-out mice for IL-6 do not develop the disease [31][32].

In the present study, the PAH model showed the characteristic damages of a previously reported model [33][34][35]. Thus, MCT induced RV hypertrophy and increased the arteriolar medial wall thickness. In this field, evidence suggests that pyrrolic derivatives of MCT that are metabolized in the liver induce pulmonary arterial endothelial cell (PAEC) damage through the activation of extracellular calcium-sensing receptors of PAECs, particularly its extracellular domain, which has the potential basic structure for MCT binding [36].

On the other hand, previous studies have demonstrated that allicin induces an anti-inflammatory effect [37][38]. It has been reported that allicin exerts an immune modulatory effect on intestinal epithelial cells through TNF-α inhibition [22]. In addition, allicin ameliorates the progression of osteoarthritis by decreasing TNF-α, IL-6, and IL-1β in chondrocytes [39]. In PAH, TNF-α, IL-6, and IL-1β lead to pulmonary arterial remodeling as they can cause damage in pulmonary endothelial cells, promoting abnormal PASMCs migration and proliferation [40][41]. Evidence also shows that the main feature of MCT-induced PAH is the infiltration of inflammatory cells and the secretion of inflammatory cytokines [42].

In this study, MCT administration increased the TNF-α, IL-6, and IL-1β levels in the lungs of rats with MCT-induced PAH. Furthermore, we found that MCT increased the expression of *Cd68*, an important macrophage marker [43]. Studies have shown that $CD68^+$ levels are increased in experimental and clinical PAH, indicating cellular inflammation, which implies an increase in the number of perivascular macrophage infiltrations [44]. In addition, a recent study found that $CD68^+$ macrophages are associated with the development of PAH [45]. Interestingly, allicin treatment prevented increases in the expressions of TNF-α, IL-6, IL-1β, and $Cd68^+$ in the MCT model. These findings suggest that allicin reduces macrophage infiltration in the lung of rats with MCT-induced PAH and, consequently, ameliorates vascular remodeling. This could be supported by the immunohistochemistry analysis of TNF-α, which showed a low production of this cytokine in the MCT group treated with allicin.

To elucidate the possible anti-inflammatory mechanism of allicin on PAH, we studied the expression of NFκB, a transcription factor that has a key role in the expression of multiple genes associated with inflammation, proliferation, and apoptosis [46]. The activation of NFκB in cytoplasm is a consequence of Iκβ inhibitory protein phosphorylation and subsequent degradation by the proteasome. NFkB can migrate to the nucleus to induce the expression of cytokines (TNF-α, IL-6, and IL-1β), as well as proteins associated with cell proliferation and apoptosis, resulting in the development of PAH. Thus, to determine the mechanism through which allicin prevents increases in TNF-α, IL-6, IL-1β, and $CD68^+$, we assessed the expression of NFκB in lung tissue. The result indicated that the protein expression levels of NFκB were lower in the allicin group than in the MCT group not treated with allicin. Other studies have demonstrated that, in MCT-induced PAH, the inhibition of NFκB improved the disease by decreasing macrophage infiltration [47]. Unexpectedly, we found that the Iκβ inhibitory protein was low in the MCT model with allicin treatment in comparison with the MCT model without allicin treatment. This could be possible because the phosphorylated form was not measured in this study. Thus, the results suggest that allicin may be considered a therapeutic alternative for inflammation in PAH through the modulation of proinflammatory cytokines and inhibition of inflammatory cell recruitment.

Remodeling of the pulmonary vasculature and fibrosis play key roles in the development and progression of PAH. In this context, TGF-β, α-SMA, fibronectin, and collagen are involved in the remodeling and fibrotic process [48][49]. Moreover, in PAH, the increase in TGF-β signaling results in the proliferation and antiapoptotic response of PAECs and PASMCs and in the increase in inflammatory cytokines [13]. Therefore, we assessed the expressions of TGF-β and α-SMA in MCT-induced PAH. Our results showed that MCT administration increased TGF-β and α-SMA in the lung tissue. Likewise, these data are in line with the analysis of fibrosis in lung tissue. Our results are in line with those previously reported [50]. Similar to inflammation, in PAH, there are no drugs that target fibrosis-related signaling pathways. Thus, we assessed the effects of allicin on fibrosis. A recent study reported that allicin decreased the TGF-β expression in the serum and renal cortex of rats with diabetic nephropathy [51]. In this work, we showed that allicin prevented increases in the expressions of TGF-β and α-SMA. Therefore, the results suggest that TGF-β and α-SMA contribute to fibrosis in the vascular wall. In addition to its role as a profibrotic protein, α-SMA is the first marker of differentiation of smooth muscle cells during the remodeling of the vascular wall in PAH [52]. Thus, the upregulation of α-SMA could contribute to the muscularization of the vascular wall, the degree of vascular occlusion, and pulmonary artery medial wall thickness [53]. To the best of our knowledge, this is the first study to report the antifibrotic effect of allicin on PAH through the modulation of TGF-β and α-SMA. To support this result, we detected TGF-β using immunohistochemistry and found a lower production of this protein in the MCT group treated with allicin. Thus, the results suggest that allicin may be considered a therapeutic alternative for fibrosis in PAH.

On the other hand, miR-21-5p plays a role in the development of PAH because it regulates the expressions of BMPR2 and TGF-β [54]. BMP signaling regulates cell proliferation, differentiation, and apoptosis [55], and it is decreased in patients with PAH, as well as in MCT models [56][57]. In this field, a decrease in BMP induces activation of the TGF-β signaling pathway [58]. Parikh et al. reported that miR-21-5p is upregulated in the lungs of rats with MCT-induced PAH [59]. In agreement with these results, we found that miR-21-5p was upregulated, while the expression of *Bmpr2* was downregulated. The allicin treatment did not modify the expressions of miR-21-5p and *Bmpr2,* and did not change *Smad5*, a transcription factor of BMP. These results suggest that allicin did not affect the *Bmpr2/smad5* signaling pathway via miR-21-5p in our experimental PAH model.

Besides the increases in inflammatory and fibrotic markers in the lung tissue, the PAH MCT model developed RV hypertrophy, which is also present in many PAH patients [34]. RV hypertrophy is a determining factor in the symptoms and survival of patients with PAH and is determined via the adaptation of RV function to the increased afterload [14]. Therefore, we assessed the expressions of inflammatory markers and fibrotic proteins in the RV. TNF-α, IL-6, and TGF-β were increased in the MCT group, and were prevented by the allicin treatment. Thus, the protective role of allicin appears to be significant and extended during the development of RV hypertrophy.

On the other hand, it is well known that the primary effects of allicin may be antioxidant and that the multiple cardioprotective effects attributed to the molecule could be due to an indirect effect. Allicin can react directly with reactive oxygen species (ROS) or free radicals or can act as a substrate for glutathione synthesis. This is supported by in vivo studies, which have reported that allicin reacts with glutathione to produce S-allyl-mercapto glutathione or with L-cysteine to produce S-allyl-mercapto cysteine [60]. Moreover, allicin prevents the formation of

free radicals and lipid peroxidation through hydroxyl and peroxyl radicals scavenging by transferring its allylic hydrogen to the oxidized substrate [61]. Indirectly, through regulation of the Nrf2/keap1 pathway and its target genes, allicin increases the presence of endogenous antioxidants, such as catalase, superoxide dismutase, heme-oxygenase, and glutathione peroxidase. At the same time, allicin regulates the secretion of proinflammatory cytokines by modulating NfκB/IκB pathway signaling [62][63][64][65]. Therefore, it is possible that the anti-inflammatory and antifibrotic effects observed in PAH could be associated with the antioxidant effects of allicin via modulation of the Nrf2/keap1 pathway. This issue could be addressed in another study.

Our study has some limitations, as follows. First, the allicin treatment started immediately after a single injection of MCT. Therefore, the effects of allicin on MCT-induced PAH could be preventive rather than curative. Second, we used an allicin dose that showed antidiabetic effects in other studies. Thus, it is possible that the effects of allicin in PAH could be dose-dependent. Third, another limitation of our study is the lack of a pulmonary hemodynamics parameter (RVSP, PVR, or PAP). However, the gold standard in MCT-induced PAH is the Fulton Index (RV/LV + S), which was assessed in our experimental model of PAH and was increased in the MCT group when compared with the control group. This index was in line with the histopathology analysis. Therefore, we conclude that the model was successfully induced. Our PAH validation results are in line with other reports in the literature [33][34][35]. Finally, this study is the first to explore the anti-inflammatory and antifibrotic effects of allicin (the major active component of garlic) in MCT-induced PAH.

Several studies have reported the beneficial effects of garlic in different presentations, such as extracts, lyophilization, and pills. Allicin has demonstrated a plethora of beneficial effects [66][67][68], but the dose used in experimental models, as well as in patients, is between 10 and 40 mg/day, and no secondary effects have been described. However, the use of allicin in patients is limited and focused on triglycerides and cholesterol alterations. Therefore, it is recommended to carry out controlled studies in patients in order to document scientific evidence to support the use of allicin in PAH.

In brief, this study showed evidence that allicin has a protective effect on pulmonary arterial medial wall thickness and RV hypertrophy in MCT-induced PAH. In addition, allicin prevented increases in inflammatory and fibrotic markers, which extended to the RV. Therefore, the nutraceutical allicin can be considered a potential therapeutic option, offering simultaneous and diverse benefits. Finally, further studies are required to show alternative mechanisms that help delay the progression of the disease.

3. Conclusions

The results showed that allicin prevented the increase in pulmonary arterial medial wall thickness and RV hypertrophy in MCT-induced PAH, which was possibly mediated by its effects on inflammatory and fibrosis markers in the lung and heart tissues. Therefore, allicin is a nutraceutical offering diverse benefits and should be considered as a potential therapeutic option to delay pulmonary function decline and right ventricle hypertrophy in the progression of PAH.

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