

# AGE–RAGE Stress in the Pathophysiology of Pulmonary Hypertension and its Treatment

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Int J Angiol 2019;28:71–79.

## Abstract

Pulmonary hypertension (PH) is a rare and fatal disease characterized by elevation of pulmonary artery pressure  $\geq 25$  mm Hg. There are five groups of PH: (1) pulmonary artery (PA) hypertension (PAH), (2) PH due to heart diseases, (3) PH associated with lung diseases/hypoxia, (4) PH associated with chronic obstruction of PA, and (5) PH due to unclear and/or multifactorial mechanisms. The pathophysiologic mechanisms of group 1 have been studied in detail; however, those for groups 2 to 5 are not that well known. PH pathology is characterized by smooth muscle cells (SMC) proliferation, muscularization of peripheral PA, accumulation of extracellular matrix (ECM), plexiform lesions, thromboembolism, and recanalization of thrombi. Advanced glycation end products (AGE) and its receptor (RAGE) and soluble RAGE (sRAGE) appear to be involved in the pathogenesis of PH. AGE and its interaction with RAGE induce vascular hypertrophy through proliferation of vascular SMC, accumulation of ECM, and suppression of apoptosis. Reactive oxygen species (ROS) generated by interaction of AGE and RAGE modulates SMC proliferation, attenuate apoptosis, and constricts PA. Increased stiffness in the artery due to vascular hypertrophy, and vasoconstriction due to ROS resulted in PH. The data also suggest that reduction in consumption and formation of AGE, suppression of RAGE expression, blockage of RAGE ligand binding, elevation of sRAGE levels, and antioxidants may be novel therapeutic targets for prevention, regression, and slowing of progression of PH. In conclusion, AGE–RAGE stress may be involved in the pathogenesis of PH and the therapeutic targets should be the AGE–RAGE axis.

## Keywords

- ▶ advanced glycation end products
- ▶ cell receptor for AGE
- ▶ soluble RAGE
- ▶ pulmonary hypertension
- ▶ pathogenesis
- ▶ therapeutic targets

Pulmonary hypertension (PH) is a rare and fatal disease characterized by elevation of the mean pulmonary artery pressure  $\geq 25$  mm Hg at rest or  $\geq 30$  mm Hg with exercise and is due to remodeling of the vasculature of the pulmonary artery and increased vasoconstriction. This disease is characterized by dyspnea initially while exercising, fatigue, dizziness or fainting, chest pressure or pain, edema of ankles, legs, ascites, cyanosis, and increased heart rate. If left untreated, it results in right ventricular failure and ultimately death.<sup>1</sup> PH has been classified as pulmonary artery hypertension (PAH), and PH due to left heart disease, lung disease and/or hypoxia, unclear multifocal mechanisms, and

chronic thromboembolism.<sup>2</sup> The incidence of portopulmonary hypertension due to cirrhosis of the liver is high compared to other types of hypertension.<sup>3</sup> The annual incidence of adult PH from the period of 2003 to 2012 increased from 24.1 to 28.7 cases/100,000 population, and the annual prevalence from the period of 1993 to 2012 increased from 98.8 to 127.3 cases/100,000 population, respectively.<sup>4</sup> Except for PAH the epidemiology of PH is largely unknown.

Advanced glycation end products (AGE) and its receptor RAGE (receptor for AGE) have been implicated in the pathophysiology of numerous diseases including systemic hypertension<sup>5</sup> and carotid artery stenosis.<sup>6</sup> Based on the involvement

of the AGE-RAGE axis in various diseases, modulation of AGE and RAGE has been proposed for the treatment of diseases related to the AGE-RAGE axis.<sup>6,7</sup> Epidemiology, classification, hemodynamics, pathogenesis, the AGE-RAGE axis and treatment modalities of PH have been addressed in this review. Special attention has been given to the role of AGE-RAGE stress in the pathophysiology of PH and its treatment with reduction in AGE-RAGE stress.

## Epidemiology

The prevalence of PAH and idiopathic PAH is 15 cases and 5.9 cases/1 million adult population, respectively. The incidence of PAH is 2.4 cases/1 million adult population/year.<sup>8,9</sup> The incidence of PAH ranged from 1.1 to 7.6/1 million, and prevalence ranged from 6.6 to 26.1/1 million for European countries, like France, U.K., Ireland, and Spain.<sup>10</sup> The prevalence of PH is up to 60% in patients with left ventricular systolic dysfunction, and up to 70% in patients with isolated left ventricular diastolic dysfunction.<sup>11</sup> The incidence of PH is 20% in chronic obstructive pulmonary disease (COPD) patients with respiratory failure,<sup>12</sup> but in advanced COPD the incidence is greater than 50%.<sup>13</sup> The incidence of PH is 39% in patients with interstitial lung disease.<sup>14</sup> The incidence and prevalence of PH in chronic pulmonary thromboembolism in the Spanish population is 0.9 case/1 million/year, and 3.2 cases/1 million, respectively.<sup>15</sup> The incidence of PH after acute pulmonary embolism is 1.0 to 3.8%.<sup>16</sup> The prevalence of PH in sarcoidosis is 1 to 28%.<sup>17</sup>

## Classification of PH

The clinical classification of PH has been updated.<sup>1</sup> PH has been classified into five groups:

1. PAH that covers idiopathic, heritable, drug- and toxin-induced, associated with connective tissue diseases, HIV (human immunodeficiency virus) infection, portal hypertension, congenital systemic to pulmonary shunts, schistosomiasis, PAH responders to calcium channel blockers, persistent pulmonary hypertension of newborn syndrome, and associated with venous/capillaries (pulmonary veno-occlusive disease, pulmonary capillary hemangiomatosis).
2. PH due to left heart diseases, such as heart failure, valvular heart diseases, and congenital/acquired cardiovascular conditions, leading to post capillary PH.
3. PH associated with lung diseases and/or hypoxia that include COPD, interstitial lung diseases, and hypoxia without lung diseases.
4. PH related to chronic obstruction of pulmonary artery, such as chronic thromboembolism and other pulmonary artery obstruction.
5. PH due to unclear and/or multifactorial mechanisms that covers hematological disorders.

## Hemodynamics of PH

Pulmonary hypertension is defined as mean pulmonary arterial pressure (mPAP)  $\geq$  25 mm Hg measured by right heart catheterization, pulmonary capillary wedge pressure (PCWP)

$\leq$  15 mm Hg and pulmonary vascular resistance (PVR)  $\geq$  240 dynes/s/cm.<sup>5</sup> Doppler's echocardiography, although correlate with right ventricular systolic pressure, they are not precise and are not substitute for accurate PAP measured by cardiac catheterization.<sup>18</sup> The abnormal elevation of mPAP in isolation is not adequate to define mPAP because it can be due to an increase in cardiac output or pulmonary capillary wedge pressure (PCWP). Hemodynamically PH is classified as pre-capillary and postcapillary PH.<sup>19</sup> In precapillary PH, mPAP is  $\geq$  25 mm Hg, PCWP is  $\leq$  15 mm Hg, and PVR is  $\geq$  240 dynes/s/cm,<sup>5</sup> and cardiac output is normal or reduced. Clinically PAH, and PH due to lung diseases, chronic thromboembolism, and unclear and/or multifactorial groups belong to this class. Hemodynamically, PH due to left heart disease is classified as postcapillary PH where mPAP is  $\geq$  25 mm Hg, PCWP is  $\geq$  15 mm Hg, and PVR is  $<$  240 dynes/s/cm,<sup>5</sup> cardiac output is normal or reduced and passive transpulmonary pressure gradient (TPG; mPAP-mPCWP) is  $\leq$  12 mm Hg, and reactive TPG is greater than 12 mm Hg.

## Pathology of PH

Pulmonary hypertension is characterized by smooth muscle cells proliferation, muscularization of peripheral pulmonary arteries, and medial thickening of larger pulmonary arteries. Muscularization is associated with fibroblastosis, reduced responses to vasodilators, and formation of obliterative plexiform lesions. The pathophysiologic mechanisms of group 1 (PAH) have been studied in detail. However, the pathophysiologic mechanisms of groups 2 to 5 PH are not that well known. The histopathological studies, such as vasculopathy, hypertrophy of media, intimal hyperplasia and fibrosis, plexiform lesions, recanalization of thrombi, and thromboembolism, are similar in all the five groups irrespective of the differences in etiology. Increased vascular resistance in PAH is due to vasoconstriction, vascular remodeling, and thrombosis.<sup>20</sup> Since the etiologies are different for each group, the author would like to describe the pathophysiology of each group in short. Group 1 (PAH): in this group, distal pulmonary artery is affected. The medial hypertrophy, intimal proliferation and fibrosis, adventitial thickening, inflammatory infiltration, and plexiform and thrombotic lesions characterize lesion. The mechanisms of these pathological changes are multifactorial. Hypoxia-induced pulmonary vasoconstriction leads to luminal narrowing. Hypoxia also inhibits voltage-gated potassium channel in the smooth muscle cells of pulmonary artery leading to the opening of the voltage-gated calcium channel.<sup>21</sup> This would constrict the pulmonary artery causing dysfunction of pulmonary arterial endothelial cells resulting in decreased production of vasodilators (prostacyclin, nitric oxide [NO]), and increased production of vasoconstrictors (endothelin-1, thromboxane A<sub>2</sub>).<sup>22</sup> Pulmonary vascular remodeling is comprised of medial hypertrophy due to proliferation of smooth muscle cells of the pulmonary artery and neointimal formation due to dysfunction and proliferation of endothelial cells.<sup>23</sup> Elevated production of the adventitial matrix and reduction in proteolysis of the extracellular matrix (ECM) would also contribute in remodeling of the pulmonary

artery.<sup>22</sup> Platelets are also involved in the pathogenesis of PAH because they occlude the vessels through thrombosis and generation of NO, a vasoconstrictor.<sup>24</sup> Abnormal platelets from patients with PAH have been shown to reduce the levels of endothelial NO synthase.<sup>25</sup> Plasma serotonin concentrations are higher in patients with PAH because of abnormal platelet processing and storage. Serotonin is a vasoconstrictor and a proliferator of smooth muscle cells. Group 2: pathological changes in this group include enlarged and thick pulmonary veins, dilated pulmonary capillaries, interstitial edema, alveolar hemorrhage and the enlargement of lymphatic vessels, and lymph nodes.<sup>19</sup> There may be medial hypertrophy and intimal fibrosis in the distal pulmonary artery. Group 3: pathological changes in this group include medial hypertrophy and intimal proliferation of the distal pulmonary artery.<sup>19</sup> Group 4: pathological lesions in this group are characterized by organized thrombi attached to inner pulmonary arterial wall, plexiform lesions, and collateral circulation from systemic arteries.<sup>19</sup> Group 5: pathological changes in this group are heterogeneous and variable.<sup>19</sup>

### AGE-RAGE Stress in the Pathogenesis of Pulmonary Hypertension

Recently, attention has been focused on the role of AGE and RAGE and the circulating sRAGE in the pathophysiology of pulmonary hypertension. The following sections describe the AGE-RAGE axis, AGE-RAGE stress, serum/plasma levels of AGE and sRAGE, and the levels of cell-bound RAGE in patients with PH.

#### AGE-RAGE Axis

AGEs are heterogeneous groups of irreversible adducts formed by nonenzymatic glycation of proteins, lipids, and nucleic acid with reducing sugars.<sup>26,27</sup> AGE interacts with its cell-bound receptor RAGE to generate reactive oxygen species (ROS)<sup>28</sup> which in turn activates nuclear factor kappa B (NF- $\kappa$ B).<sup>29</sup> Activated NF- $\kappa$ B activates numerous genes including proinflammatory cytokines,<sup>30</sup> and adhesion molecules.<sup>31</sup> RAGE is a multiligand receptor which can bind with many ligands. There are two isoforms of RAGE: cleaved RAGE (cRAGE), and endogenous secretory RAGE (esRAGE). cRAGE is proteolytically cleaved from full length RAGE,<sup>32</sup> while esRAGE is produced from splicing of full length RAGE mRNA.<sup>33</sup> Measurements of sRAGE include both cRAGE and esRAGE. sRAGE acts as a decoy for RAGE by binding with RAGE ligand.<sup>34</sup> sRAGE binding with ligands does not activate intracellular signaling. sRAGE is cytoprotective because it protects from the adverse effects of interaction of RAGE with ligands.

#### AGE-RAGE Stress

In the AGE-RAGE axis which is comprised of AGE, RAGE, and sRAGE, AGE and RAGE are coined as stressors, while sRAGE, enzymatic degraders of AGE (glyoxalase-1, glyoxalase-2), receptor-mediated degraders of AGE (AGER-1, AGER-2), and factors that lower the blood levels of AGE have been

coined as antistressors.<sup>35</sup> The ratio of stressors/antistressors has been termed as AGE-RAGE stress.<sup>35</sup> The ratio of AGE/sRAGE has been proposed as a simple and feasible measure of AGE-RAGE stress.<sup>35</sup> A high index of AGE/sRAGE would result in tissue damage and the development of diseases.

#### Serum Levels of AGE in PH

Serum/plasma levels of AGE in patients with PH are not available in literature. However, serum/plasma levels of AGE in diseases associated with PH are known. Plasma levels of AGE (carboxymethyl lysine [CML]) are elevated in COPD patients compared with non-COPD patients.<sup>36</sup> AGE levels are elevated in the lungs of patients with COPD.<sup>37</sup> The AGE levels are elevated in the skin of COPD patients.<sup>38</sup> Serum levels of AGE are significantly increased in patients with left ventricular diastolic dysfunction in type 1 diabetes<sup>39,40</sup> and heart failure.<sup>41,42</sup>

#### Levels of RAGE in PH

The RAGE in endothelial cells of large and small pulmonary arteries, neointimal proximal remodeled pulmonary arteries, recanalized vessel-like structure of distal endarterectomized chronic thromboembolism PH (CTEPH) and idiopathic PAH (iPAH) were differently expressed.<sup>43</sup>

There is an increased expression of RAGE in smooth muscle cells of the pulmonary artery under hypoxic conditions in both humans and mice.<sup>44</sup> These authors also showed that the expression of RAGE was upregulated in pulmonary artery in hypoxia plus Sugen5416 (SU5416)-induced PAH mice. They also demonstrated that RAGE deletion reduced the PA pressure and restrained ECM accumulation in PA of the mouse model. Blocking RAGE activity with neutralizing antibody or genetic deletion of RAGE reduced ECM protein accumulation.<sup>44</sup> RAGE is one of the most upregulated proteins in PAH lung tissue of PAH patients.<sup>45</sup> There is an increase in the RAGE mRNA levels in human pulmonary hypertensive lung tissue compared with normotensive lung tissue, and an increase in RAGE protein levels in pulmonary artery in human pulmonary hypertensive patients compared with normotensive pulmonary artery (five-fold increase).<sup>46</sup> They also showed that that there was no upregulation of RAGE in other tissue (brain, kidney, and peripheral muscle). Increased RAGE activation by its ligands including AGE increases RAGE expression.<sup>28</sup> It is possible that the increases in AGE in PH could increase the expression of RAGE. The data suggest that RAGE levels are elevated in patients with PH.

#### Serum/Plasma Levels of sRAGE in PH

Serum levels of sRAGE are variable in PH. Plasma levels of sRAGE were significantly lower in patients with idiopathic pulmonary fibrosis and interstitial lung disease.<sup>47</sup> Serum sRAGE levels are reduced in patients with emphysema.<sup>48</sup> Serum levels of sRAGE were lower in patients with COPD than the levels in control.<sup>38</sup> However, there are reports where sRAGE levels have been higher in PH patients compared with

controls. Plasma levels of sRAGE have been reported to be higher in patients with PH and CTEPH than those in controls.<sup>49</sup> Serum levels of sRAGE and esRAGE were higher in patients with iPAH and CTEPH compared with control subjects.<sup>43</sup> The discrepancy in the serum levels of sRAGE might be due to the type of patients. High levels of sRAGE were in patients with pulmonary hypertension, while the low levels of sRAGE were in patients with lung diseases without hypertension.

### Mechanism of AGE-RAGE-Induced Pulmonary Hypertension

Remodeling of pulmonary artery in PAH is due to enhanced proliferation and resistance to apoptosis of pulmonary artery smooth muscle cells. Since the histopathology of PH includes intimal hyperplasia due to an increase in the proliferation of smooth muscles and fibrosis, the focus will be on the role of AGE, RAGE, and sRAGE on smooth muscle proliferation and fibrosis. Attention will also be given to the vasoconstrictor effect of the AGE-RAGE axis. Stiffness of the pulmonary artery is due to the increased amounts of collagen, glycation of collagen, and cross-linking of collagen with AGEs. AGEs are formed in the proteins of ECM. Accumulation of AGE on protein of ECM leads to the formation of cross-link which traps other local macromolecules.<sup>50</sup> Cross-linking of collagen and elastin increases the ECM area which increases the stiffness of the artery.<sup>51</sup> Glycation also increases the synthesis of collagen.<sup>52</sup> There is cross-linking of elastin with AGE which reduces the elasticity of arteries. Matrix-bound AGE inhibits antiproliferative activity of NO,<sup>53</sup> reduce half-life of NO synthase,<sup>54</sup> impair NO production,<sup>55</sup> inactivates NO,<sup>56</sup> reduces the production of prostacycline,<sup>57</sup> and increases the expression of endothelin-1.<sup>58</sup> AGE promotes proliferation and suppresses autophagy via reduction in cathepsin D in vascular smooth muscle cells.<sup>59</sup> AGE promotes proliferation and migration of primary rat vascular smooth muscle cells via oxidative stress.<sup>60</sup> The above data suggest that AGE can increase thickness and resistance of the pulmonary artery through increasing the matrix of blood vessels.

As described earlier, RAGE is a multiligand receptor and combines with ligands, such as AGE, high mobility group box-1 (HMGB-1), and calcium binding calgranulin-like protein S100A4 (S100). S100 protein family consists of 24 members. Extracellular S100A4 is one of the 24 members of S100. RAGE has been implicated to play a role in many signaling pathways, such as inflammation, proliferation, and migration, all of which are associated with pathology of PH.<sup>46</sup> The AGE-RAGE interaction leads to the generation of ROS, and the proliferation and autophagy in vascular smooth muscle cells.<sup>61</sup> In vitro, S100A4 produces proliferation of human pulmonary arterial smooth muscle cells via an action non-RAGE.<sup>62</sup> RAGE mediates deposition of ECM fibronectin and collagen through activation of transforming growth factor- $\beta$ 1.<sup>44</sup> Vascular remodeling of the PA in PAH is characterized by deposition of ECM.<sup>63</sup> Binding of RAGE with HMGB-1 induces proliferation and migration of fibroblasts which are prevented by RAGE antibody.<sup>64</sup> RAGE regulates the metabolic reprogramming-induced over-proliferation in pulmonary hypertension.<sup>65</sup> Recently, it has been

shown that RAGE plays a crucial role in inappropriate increase in pulmonary arterial smooth muscle cells in PAH.<sup>66</sup> These above data suggest that AGE and its interaction with RAGE would muscularize the pulmonary artery, induce medial thickness and fibrosis, and increase stiffness in the pulmonary artery.

AGE-RAGE interaction increases the generation of reactive oxygen species including Superoxide anion, hydrogen peroxide, and hydroxyl radicals. Superoxide anion produces contraction of isolated rabbit aorta which is endothelium-dependent and is partially mediated by arachidonic acid metabolism.<sup>67</sup> H<sub>2</sub>O<sub>2</sub> in lower concentration produces contraction, while in higher concentration, it produces transient relaxation followed by contraction of isolated rabbit aorta.<sup>68</sup> In vivo, ROS generated by polymorphonuclear leucocytes and administration of oxygen radicals increase total peripheral vascular resistance.<sup>69,70</sup> Oxidative stress plays a key role in the pathogenesis of pulmonary components of COPD.<sup>71</sup> COPD patients have levels of ROS in plasma and endothelial cells compared with control subjects. Emerging evidence demonstrates the role of ROS in PH pathology. ROS modulates cellular proliferation and attenuates apoptosis.<sup>72</sup> ROS plays a role in mediating vasoconstrictor reactivity and pulmonary hypertension in both chronic hypoxia and hypoxia/SU5416 rat model.<sup>73</sup> AGE-RAGE-induced generation of ROS increases the pulmonary arterial pressure directly and through hypertrophy of pulmonary artery. The above data suggest that AGE and its interaction with RAGE play a crucial role in pathogenesis of PH.

### Therapeutic Approaches Targeting AGE-RAGE Axis for Pulmonary Hypertension

Current treatment of pulmonary hypertension has progressed due to pulmonary hypertension-targeted drugs. However, long-term survival of patients with PAH is still suboptimal. A search for new treatment modalities that can reverse pulmonary artery remodeling is on. Lately, attention has been focused on the AGE-RAGE axis because it is involved the pathogenesis of PH. AGE-RAGE axis (AGE, RAGE, and sRAGE) is an important therapeutic target. Reduction in AGE and RAGE levels, elevation of sRAGE levels and antioxidant are promising new therapeutic strategies in PH.

#### Reduction in the AGE levels

AGE levels in the body can be lowered by reducing dietary consumption of AGE, preventing AGE formation and increasing AGE degradation.

#### Reduction in Dietary Consumption of AGE

Glucose consumption should be reduced because glucose is involved in the synthesis of AGE.<sup>27</sup> Consumption of high AGE-rich diets<sup>74</sup> including, red meat, cheese, cream, butter, and animal fat those have higher amounts of AGE than oil and nuts should be reduced. Consumption of diets, such as butter, cream, cheese, margarine, and mayonnaise, those have highest amount of AGE should also be reduced.<sup>75</sup> These authors also reported that beef has the highest amount of AGE,

followed by poultry, pork, fish, and eggs. Diets with the lowest amount of AGE,<sup>75</sup> such as grains, legumes, vegetables, fruits, and milk, should be consumed.<sup>75</sup> Fat-free milk has lower amounts of AGE than whole milk. Cooking at high temperatures in dry heat should be avoided or markedly reduced because it increases the formation of AGE. Frying, broiling, grilling, and roasting generates more AGE than poaching, stewing, steaming, and boiling.<sup>75</sup> There is a reduction in AGE formation when cooking for short duration in moist heat at low temperature.<sup>75</sup> Short-term restriction of dietary AGE has been reported to significantly reduce the serum levels of AGE in healthy or diabetic individuals.<sup>76</sup> Hence, foods should not be cooked at high temperature in dry heat. Cigarette smoking should be stopped because it increases serum levels of AGE.<sup>77</sup>

### Prevention of AGE formation

There are numerous vitamins which inhibit the formation of AGE. Benfotiamine (vitamin B1 derivative),<sup>78</sup> pyridoxine,<sup>79</sup> vitamins C,<sup>80</sup> D,<sup>81</sup> and E<sup>82</sup> prevent the formation of AGE. Use of these vitamins may help the patients with PH. Carnosine inhibits the formation of AGE through acting as an antioxidant,<sup>83</sup> a chelating agent for metal ions,<sup>84</sup> and reacting with the carbonyl group of methylglyoxal (MG)-modified proteins resulting in protein-carbonyl-carnosine adducts. Carnosine modified AGE becomes ineffective to interact with RAGE.<sup>85</sup> Acidic ingredients, such as lemon juice or vinegar,<sup>86</sup> and pomegranate and its phenolic components<sup>87</sup> inhibit the formation of AGE. The patients with PH should be advised to consume the above agents.

### Degradation of AGE

AGE is degraded in the body in two ways: enzymatic degradation and AGE-receptor-mediated degradation. Glyoxalase-1 and glyoxalase-2 degrade AGE.<sup>88</sup> Overexpression of glyoxalase-1 in bovine endothelial cells completely prevented hyperglycemia-induced AGE formation.<sup>89</sup> AGE receptor mediated degradation of AGE is accomplished by AGE receptor-1 (AGER-1), AGER-2, and AGER-3.<sup>35,90</sup> AGER-1 increases the uptake and removal of AGE and blocks AGE-RAGE mediated production of ROS and cytokines.<sup>90</sup> Drugs should be developed to overexpress the above enzymes and receptors to reduce the levels of AGE.

### Antagonist of RAGE

RAGE is antagonized by the suppression of its expression, blockage of ligand binding, and inhibition of RAGE signal transduction to antagonize RAGE receptor.

### Downregulation of RAGE expression

Lipid lowering agents (simvastatin,<sup>91</sup> and atorvastatin<sup>92</sup>), angiotensin-II receptor blockers (telmisartan<sup>93</sup> and candesartan<sup>94</sup>), antidiabetic agent (thiazolidinediones<sup>95</sup>), calcium channel blocker (nifedipine<sup>96</sup>), and curcumin<sup>97</sup> downregulate the RAGE expression. Most of the above-mentioned drugs except curcumin are being used for the treatment of certain diseases and will have additional benefits. Meloche et al<sup>46</sup> has reported that RAGE inhibition by RAGE siRNA

selectively delivered to the lungs of monocrotaline and Sugen-hypoxia-induced pulmonary hypertension in rats reversed the pulmonary hypertension. This shows that suppression of RAGE expression can indeed reverse the pulmonary hypertension.

### Blockers of RAGE Ligand Binding

Recently new drugs have been developed to block RAGE ligand binding for the treatment of Alzheimer's disease. The drugs TTP488 or PF-04494700, also known as Azeliragon, and TTP4000 prevent RAGE ligand from interacting with RAGE.<sup>98</sup> These new drugs has been shown to be of benefit in patients with Alzheimer's disease. These drugs have not been tried in patients with PH. Inhibitors of RAGE have been discussed in detail by Bongarzone et al.<sup>99</sup>

### sRAGE

RAGE activation may be prevented by sRAGE which competes with RAGE for the same ligands and therefore fewer amounts of ligands are spared to interact with RAGE to activate intracellular signaling and produce harmful effects. sRAGE can prevent RAGE signal transduction directly by preventing the homodimerization of RAGE on cell surface binding with ligands and does not activate intracellular signaling.<sup>100</sup>

### Raising the Levels of sRAGE

Since sRAGE competes with RAGE for AGE ligand and since interaction of sRAGE with AGE does not activate intracellular signaling, raising the levels of sRAGE would have beneficial effects in patients with PH. Certain drugs commonly used in patients for cardiovascular diseases and diabetes raise the levels of sRAGE. Statins (pitavastatin and pravastatin<sup>101</sup>) in humans; (atorvastatin, fluvastatin, and lovastatin<sup>102</sup>) in isolated cell lines; angiotensin converting enzyme inhibitors ramipril<sup>103</sup> in serum of diabetic rats and perindopril<sup>104</sup> in serum of type-1 diabetics; and the antidiabetic drug, rosiglitazone, elevated the levels of sRAGE. Vitamin D elevates the serum levels of sRAGE in women with PCOS.<sup>105</sup> Exogenous administration of sRAGE has been demonstrated to reduce/reverse RAGE-mediated pathology in the animal model. sRAGE administration exogenously suppressed the development of atherosclerosis and restenosis, prevented destabilization of vulnerable plaques and reduced ischemia-reperfusion-induced myocardial injury.<sup>26</sup> Park et al<sup>106</sup> reported that sRAGE totally prevented the development of atherosclerosis in apolipoprotein-E (ApoE)-deficient mice independent of glycemia and lipids. sRAGE markedly reduced the carotid artery restenosis in mice,<sup>107</sup> and AGE-induced vasculopathy in diabetic rats.<sup>108</sup> Administration of sRAGE either peripherally or directly to organs has been shown to reverse some of RAGE-mediated pathological effects in vivo.<sup>109</sup> sRAGE given intraperitoneally in the dose of 20 microgram/day for 14 days in hypoxia-induced PH in mice reduced hypoxia-induced PH, right ventricular systolic pressure and peripheral vascular resistance but did not affect distal pulmonary vascular remodeling.<sup>110</sup> These data suggest that sRAGE could prevent, regress, and slow the progression of PH.

## Antioxidants

As mentioned earlier,<sup>72,73</sup> ROS plays a major role in the development of PH. Considering that antioxidants may be an option for the treatment of PH. In experimental studies, it has been shown that antioxidants attenuate the remodeling of pulmonary vasculature.<sup>111,112</sup> In a clinical trial, melatonin administered in the dose of 3 mg/kg, orally for 3 months to patients with moderate to severe COPD, reduced oxidative stress, and improved dyspnea.<sup>113</sup> Melatonin suppressed hypoxia-induced PH and reduced the proliferation of smooth muscle cells of pulmonary artery in rats.<sup>114</sup> Melatonin (6 mg/kg) administered orally to monocrotaline treated rats (model of pulmonary hypertension), improved lung edema, reduced right ventricular (RV) hypertrophy and improved RV function and reduced interstitial cardiac and lung fibrosis, and oxidative stress.<sup>115</sup> Details of melatonin for possible treatment of PH are given by Maarman.<sup>115</sup> There are numerous enzymatic and other nonenzymatic oxidants that can be of value in the adjunct therapy of PH. Attention should be given to some of the vitamins, such as vitamins E, C, and D, for use as antioxidants in PH patients. These vitamins have some beneficial effects in patients with Alzheimer's disease.

## Discussion

Serum/plasma levels AGE have not been measured in patients with PH. However the levels are elevated in the diseases where PH occurs.<sup>36–42</sup> It would be useful if the levels of AGE were measured because this would help in the assessment of AGE–RAGE stress which is measured as AGE/sRAGE.<sup>35</sup> This ratio is a risk factor for disease and a high ratio indicates the presence of disease and its complications.<sup>35</sup> Levels of RAGE have been reported to be elevated in patients with PH.<sup>44–46</sup> Interaction of elevated levels of AGE and RAGE would increase the production of ROS, proinflammatory cytokines, and vascular remodeling. Serum levels of sRAGE are variable in patients with PH and associated diseases. Plasma levels of sRAGE have been reported to be higher in patients with PH,<sup>43,49</sup> while the levels are lower in patients with lung disease without PH<sup>38,47,48</sup> as compared with controls. As stated earlier, sRAGE is cytoprotective and hence, high levels of sRAGE would have protected the development of PH but it did not do so. This is possible because both AGE and sRAGE are elevated in patients with PH but the elevation of AGE may be greater than the elevation of sRAGE. If this happens then more AGE is available to interact with RAGE to induce PH and its complication. Recently Prasad<sup>116,117</sup> has reported that AGE/sRAGE, but not AGE or sRAGE individually, is a risk factor/biomarker of diseases. Why not use the levels of RAGE also in this equation? RAGE levels can be measured in animal studies but in humans, it is not possible to measure cell receptor RAGE. For measurement of RAGE one has to get tissue samples. As stated earlier, AGE–RAGE stress has been defined as a shift in the balance between stressors (AGE, RAGE) and antistressors (AGE degraders, sRAGE) in favor of stressors. A simple and feasible

measure of AGE–RAGE stress (AGE/sRAGE) for clinical practice has been developed by Prasad and Mishra.<sup>35</sup> A high ratio of AGE/sRAGE indicates the presence of disease and its complication.

From the available data, it appears that AGE–RAGE stress is involved in the pathogenesis of PH. To date, the studies that have been performed are mostly related to RAGE in the pathogenesis of PH. The serum levels of AGE and sRAGE should be measured in the same patient so that AGE-stress can be assessed. Measurement of AGE or sRAGE individually does not provide a complete picture in the pathogenesis of PH. Plasma levels of AGE and sRAGE, and tissue levels of RAGE should be measured in animal models of PH to provide a role of AGE–RAGE stress in the pathogenesis of PH. A robust clinical trial is needed for identifying the role of AGE–RAGE stress in the pathogenesis of PH.

Considering the involvement of the AGE–RAGE axis in the development of PH, the treatment should include reduction in AGE and RAGE levels and elevation of sRAGE levels in PH patients which have been discussed earlier in detail by the author in this manuscript. Reduction of AGE levels should be an adjunct therapy along with any other treatments for PH. Consumption of acidic food, vitamins B1, B6, C, D, and E should be encouraged because these agents attenuate the synthesis of AGE. New drugs should be developed to block RAGE ligand binding. Human recombinant sRAGE should be developed for human use. Use of antioxidant vitamins, especially vitamins C and E should not be ignored. Combined use of drugs that reduce AGE levels and expression of RAGE, blocker of RAGE ligand binding, and elevation of sRAGE levels would block all pathways of the AGE–RAGE axis and hence, would be more effective than a single pathway blocker. While remembering that AGE–RAGE stress may not be the only risk factor for PH, one should not expect that.

## Conclusions

The data, to date, suggest that AGE–RAGE stress may be involved in the pathogenesis of PH. AGE and its interaction with RAGE induce pulmonary vascular hypertrophy through proliferation of smooth muscle cells, accumulation of ECM and suppression of autophagy. AGE–RAGE-induced increase in ROS modulates cellular proliferation, attenuates apoptosis, and constricts the blood vessels. Increased stiffness of the artery due to vascular hypertrophy and vasoconstriction results in pulmonary hypertension. The data also suggest that the reduction in consumption and formation of AGE, suppression of RAGE expression, blockage of RAGE ligand binding, elevation of sRAGE, and antioxidant may be a novel therapeutic target for prevention, regression, and slowing of progression of PH.

### Disclosure

None.

### Conflict of Interest

None.

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