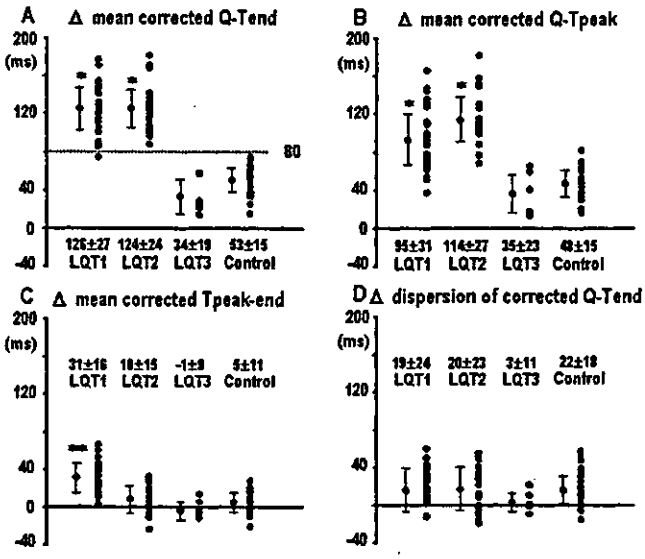

Figure 4 Composite data of changes (Δ) in the mean corrected Q-Tend (**A**), Q-Tpeak (**B**), Tpeak-end (**C**), and dispersion of corrected Q-Tend (**D**) between baseline conditions and peak epinephrine effects in LQT1, LQT2, LQT3, and Control groups of the prospective study. $*P < .05$ vs LQT3 and Control; $**P < .05$ vs LQT2, LQT3 and Control.

cally prolonged at peak epinephrine effect (503 ± 33 to 627 ± 30 ms, 411 ± 26 to 525 ± 32 ms; $P < .05$, respectively) in the LQT2 group but returned to baseline levels at steady state (518 ± 38 ms, 424 ± 36 ms; P = NS vs baseline, respectively; Figure 3A and 3B, open circles). The mean corrected Tpeak-end was unchanged with epinephrine (92 ± 23 to 102 ± 18 to 94 ± 19 ms) in the LQT2 group (Figure 3C, open circles). The mean corrected Q-Tend and Q-Tpeak were much less prolonged at peak epinephrine effect (LQT3: 506 ± 41 to 540 ± 28 ms; P = NS, 432 ± 40 to 467 ± 26 ms; P = NS, Control: 408 ± 19 to 461 ± 19 ms, 332 ± 17 to 380 ± 23 ms; $P < .05$, respectively) in the LQT3 and Control groups than in the LQT1 or LQT2 group and were shortened to the baseline levels at steady state (LQT3: 496 ± 37 ms, 427 ± 30 ms; Control: 415 ± 18 ms, 333 ± 19 ms; P = NS vs baseline, respectively) (Figure 3A and 3B, closed triangles and open triangles). The mean corrected Tpeak-end was unchanged with epinephrine (LQT3: 74 ± 7 to 73 ± 4 to 69 ± 10 ms; Control: 75 ± 8 to 81 ± 13 to 82 ± 11 ms) in the LQT3 and Control groups (Figure 3C, closed triangles and open triangles). The dispersion of corrected Q-Tend was increased at peak epinephrine effect in the LQT1 and Control groups (LQT1: 61 ± 21 ms, 79 ± 27 ms; Control: 40 ± 14 ms, 63 ± 19 ms; $P < .05$, respectively).

Figure 4 illustrates the changes (Δ) in the ECG parameters between baseline conditions and peak epinephrine effects in the four groups of the prospective study. Both the Δ mean corrected Q-Tend and Q-Tpeak were no different between the LQT1 and LQT2 groups, but they were significantly greater than those in the LQT3 and Control groups ($P < .05$; Figure 4A and 4B). No significant differences were observed in the Δ mean corrected Q-Tend and Q-Tpeak between the LQT3 and Control groups. The Δ mean corrected Tpeak-end was significantly greater in the LQT1 group than in the other three groups ($P < .05$; Figure 4C). No significant differences were observed in the Δ dispersion of corrected Q-Tend among the four groups (Figure 4D). As suggested by the retrospective study, the Δ mean corrected Q-Tend ≥80 ms at peak epinephrine effect could most effectively differentiate the LQT1 and LQT2 groups from the LQT3 or Control group (Figure 4A).

Figure 5 illustrates Δ in the ECG parameters between baseline conditions and steady-state epinephrine effects in the four groups of the prospective study. The Δ mean corrected Q-Tend, Q-Tpeak, and Tpeak-end were significantly greater in LQT1 than in the other three groups ($P <$ .05; Figure 5A–5C). The Δ mean corrected Q-Tend was significantly larger in the LQT2 than in LQT3 group ($P <$ .05; Figure 5A). There were no significant differences in the Δ dispersion of corrected Q-Tend among the four groups (Figure 5D). As suggested by the retrospective study, the Δ mean corrected Q-Tend ≥35 ms at steady-state epinephrine effect could most effectively differentiate the LQT1 group from the other three groups (Figure 5A).

Improvement of clinical diagnosis with epinephrine test

The sensitivity (i.e., penetrance) and specificity for identifying genotype-positive LQT1, LQT2, and LQT3 patients by the ECG diagnostic criteria before and after steady-state epinephrine effects were evaluated in the prospective study.

The sensitivity for identifying genotype-positive LQT1 patients among the LQT1 and Control groups was low under baseline conditions; 68% (21/31) using the ECG

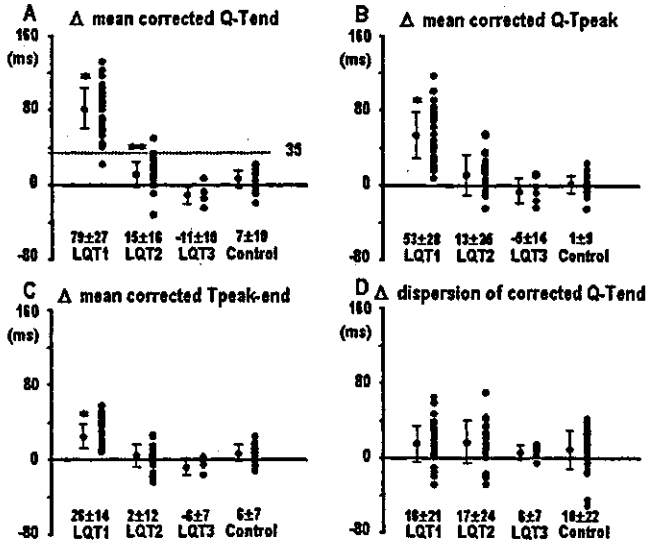

Figure 5 Composite data of changes (Δ) in the mean corrected Q-Tend (**A**), Q-Tpeak (**B**), Tpeak-end (**C**), and dispersion of corrected Q-Tend (**D**) between baseline conditions and steady-state epinephrine effects in LQT1, LQT2, LQT3 and Control groups of the prospective study. $*P < .05$ vs LQT2, LQT3 and Control; $**P < .05$ vs LQT3.

Table 2 Prediction of genotype with the epinephrine test in prospective study

	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Accuracy
LQT1 vs LQT2	97%	96%	97%	96%	96%
Δ Mean corrected Q-Tend ≥35 ms (Steady state-Baseline)	(90%)	(83%)	(88%)	(86%)	(87%)
LQT1 vs LQT3	97%	100%	100%	86%	97%
Δ Mean corrected Q-Tend ≥35 ms (Steady state-Baseline)	(90%)	(100%)	(100%)	(67%)	(92%)
LQT1 vs control	97%	100%	100%	97%	98%
Δ Mean corrected Q-Tend ≥35 ms (Steady state-Baseline)	(90%)	(97%)	(97%)	(91%)	(93%)
LQT2 vs LQT3	100%	100%	100%	100%	100%
Δ Mean corrected Q-Tend ≥80 ms (Peak-Baseline)	(91%)	(100%)	(100%)	(75%)	(93%)
LQT2 vs control	100%	100%	100%	100%	100%
Δ Mean corrected Q-Tend ≥80 ms (Peak-Baseline)	(91%)	(90%)	(88%)	(93%)	(91%)

Percentages in parentheses indicate those calculated by data measured simply from ECG lead V_5. Δ-Increase with epinephrine.

diagnostic criteria, 68% (21/31) when an LQTS score ≥4 was used, and 74% (23/31) when a score ≥2 was used. The specificity was 100% (30/30) regardless of the criteria. The sensitivity was substantially improved by measurement of the mean corrected Q-Tend at steady-state epinephrine effect without the expense of specificity (100% [30/30]); 87% (27/31), 81% (25/31), and 90% (28/31), respectively.

The sensitivity for identifying genotype-positive LQT2 patients among the LQT2 and Control groups was relatively high under baseline conditions; 83% (19/23), 83% (19/23), and 96% (22/23), respectively. The sensitivity was further improved at steady-state epinephrine effect to 91% (21/23), 91% (21/23), and 96% (22/23), respectively, without the expense of specificity (100% [30/30]).

The sensitivity for identifying genotype-positive LQT3 patients among the LQT3 and Control groups under baseline conditions was 83% (5/6), 50% (3/6), and 100% (6/6), respectively, which was unchanged at steady-state epinephrine effect by any of the three criteria.

Prediction of genotype with epinephrine test

Table 2 illustrates the predictive values with the epinephrine test for genotyping in the prospective study. The Δ mean corrected Q-Tend ≥35 ms at steady-state epinephrine effect could differentiate LQT1 from the LQT2, LQT3, or Control group with predictive accuracy ≥90%. The Δ mean corrected Q-Tend ≥80 ms at peak epinephrine effect could differentiate LQT2 from LQT3 or Control group with predictive accuracy of 100%. Even if we calculated the predictive values by the Δ corrected Q-Tend, which was measured simply from ECG lead V_5, the predictive accuracy still was high (≥80%).

At molecular screening, the responsible mutations could be identified in the first targeted gene suspected by the epinephrine test in all of the 12 LQT1, 12 LQT2, and 3 LQT3 families of the prospective study.

Response to epinephrine test in genotype-unknown patients

Figure 6 illustrates Δ mean corrected Q-Tend at peak (Figure 6A) and steady-state (Figure 6B) epinephrine effects in the 29 patients (15 probands and 14 family members) of the prospective study in whom the responsible mutations could not be identified in any LQTS genes. Among the 15 probands, the response to the epinephrine test was LQT1 pattern in 11 probands and LQT2 pattern in 4 probands. Among the 14 family members, the response was LQT1 pattern in 3 members, LQT2 pattern in 3 members, and LQT3 or Control pattern in 8 members. Even though these 29 patients without causative mutations were included in the analysis for genotype prediction, the positive predictive values were 67% (30/31+14) for LQT1 syndrome and 73% (22/23+7) for LQT2 syndrome, respectively.

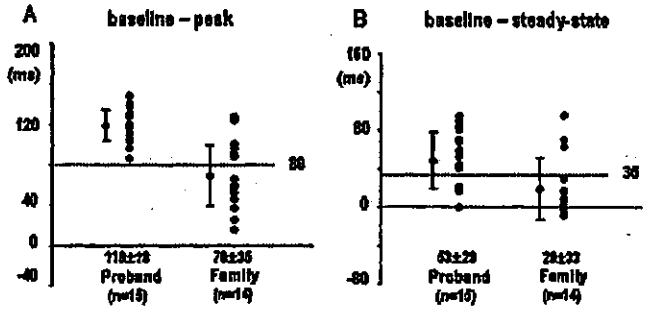

Figure 6 Composite data of changes (Δ) in the mean corrected Q-Tend between baseline conditions and peak epinephrine effects (**A**) and between baseline conditions and steady-state epinephrine effects (**B**) in the 29 patients (15 probands and 14 family members) of the prospective study in whom the responsible mutations could not be identified in any LQTS genes.

Complications

Spontaneously terminating torsades de pointes was induced by epinephrine infusion in one LQT1 patient, and spontaneous premature ventricular contractions were induced in one LQT1 and two LQT2 patients.

Discussion

The main findings of the present study are as follows: (1) penetrance in the absence of sympathetic stimulation was lower in LQT1 than in LQT2 or LQT3 syndrome and was improved with steady-state epinephrine in LQT1 and LQT2, but not in LQT3 syndromes; and (2) epinephrine infusion was a powerful test to predict the genotype of LQT1, LQT2, and LQT3 syndromes by comparing the Δ corrected Q-Tend at peak and steady-state epinephrine effects.

Penetrance in LQT1, LQT2, and LQT3 syndromes

It has long been expected that all genotype-positive patients could not be diagnosed by using ECG diagnostic criteria.[17,18] Priori et al[19] conducted molecular screening in nine families with sporadic cases of LQTS and suggested that clinical diagnostic criteria had low sensitivity (penetrance; 38%) in identifying mutation carriers. Swan et al[20] reported that the sensitivity and specificity for identifying genotype-positive patients were 53 and 100%, respectively, in a LQT1 family (D188N). Similarly, in the 12 LQT1 families of the prospective study, the sensitivity for identifying LQT1 patients was low under baseline conditions and was substantially improved with the epinephrine test without the expense of specificity. In contrast, the sensitivity for identifying LQT2 and LQT3 patients was relatively high under baseline conditions in the 12 LQT2 and 3 LQT3 families. These findings suggest the need for molecular screening of all family members regardless of clinical diagnosis to confirm genotype-positive patients, especially in LQT1 syndrome.

Epinephrine test for predicting genotype of LQT1, LQT2, and LQT3 syndromes

Recent clinical data on genotype-phenotype correlation and experimental data in LQTS models have demonstrated the genotype-specific response to sympathetic stimulation and the possibility of genotype-specific therapy.[5–8,11–14,21–23] The LQT1, LQT2, and LQT3 syndromes constitute approximately two thirds of genotyped LQTS patients.[24] Therefore, genotyping of the three forms as well as identifying latent genotype-positive patients are of particular importance in the management and treatment of LQTS patients. Because molecular diagnosis still is unavailable to many institutes, is costly, and is time consuming, genotype identification by clinical tests would be useful for stratifying molecular screening by targeting suspected genes for an initial study.[25–28] Moreover, there are still 30% to 40% of patients clinically affected with LQTS in whom no responsible mutations can be identified. Therefore, it is of great importance to diagnose, based on clinical findings, the form of LQTS that patients are affected with.

Our data demonstrate that epinephrine infusion enables us to predict the genotype of LQT1, LQT2, and LQT3 syndromes as well as to improve the clinical diagnosis of genotype-positive patients, especially in LQT1 syndrome. Genotype prediction of the three syndromes by the epinephrine test would facilitate molecular screening by targeting suspected genes. In fact, molecular screening identified the responsible mutations in the first targeted gene suspected by the epinephrine test in all of the 12 LQT1, 12 LQT2, and 3 LQT3 families of the prospective study. On the other hand, the other 15 probands were assigned to a likely genotype by the epinephrine test, but no mutations were found in any LQTS genes. Because the response to the epinephrine test was LQT1 (11 probands and 3 family members) or LQT2 pattern (4 probands and 3 family members), some ion channel or membrane adapter genes, which are sensitive to catecholamines, may be candidates for responsible genes. It is noteworthy that the positive predictive values for LQT1 and LQT2 syndromes still were high (67% for LQT1 and 73% for LQT2), even though the 29 patients without responsible mutations in any LQTS genes were included in the analysis for genotype prediction. The genotype prediction also may help to stratify the management and treatment of LQTS patients, if the patients cannot be genotyped by the molecular screening.

Conclusion

Epinephrine infusion is a powerful test to predict the genotype of LQT1, LQT2, and LQT3 syndromes as well as to improve the clinical diagnosis of genotype-positive patients, especially in LQT1 syndrome.

Acknowledgments

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Review

Adrenomedullin in the treatment of pulmonary hypertension

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Abstract

Adrenomedullin (AM) is a potent, long-lasting pulmonary vasodilator peptide. Plasma AM level is elevated in patients with primary pulmonary hypertension (PPH), and circulating AM is partially metabolized in the lungs. These findings suggest that AM plays an important role in the regulation of pulmonary vascular tone and vascular remodeling. We have demonstrated the effects of three types of AM delivery systems: intravenous administration, inhalation, and cell-based gene transfer. Despite endogenous production of AM, intravenously administered AM at a pharmacologic level decreased pulmonary vascular resistance in patients with PPH. Inhalation of AM improved hemodynamics with pulmonary selectivity and exercise capacity in patients with PPH. Cell-based AM gene transfer ameliorated pulmonary hypertension rats. These results suggest that additional administration of AM may be effective in patients with pulmonary hypertension. AM may be a promising endogenous peptide for the treatment of pulmonary hypertension.

Keywords: Adrenomedullin; Pulmonary hypertension; Inhalation; Gene therapy

Contents

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1. Introduction

Primary pulmonary hypertension (PPH) is a rare but life-threatening disease characterized by progressive pulmonary hypertension, ultimately producing right ventricular failure, and death [42,43]. Median survival is considered to be 2.8 years from the time of diagnosis. Because the presence of endothelial injury in the pulmonary vascular bed develops pulmonary vasoconstriction, smooth muscle cell proliferation, and in situ thrombosis [1], a variety of vasodilators, anti-proliferative agents, and anticoagulants have been proposed as therapeutic agents of PPH [3,10,23,45]. Despite therapeutic medical advances including prostacyclin therapy [3,23,45], some patients ultimately require heart–lung or lung transplantation [38,41]. Thus, a novel therapeutic strategy is desirable for the treatment of pulmonary hypertension including PPH.

Adrenomedullin (AM) is a potent, long-lasting vasodilator peptide that was originally isolated from human pheochromocytoma [19]. The peptide consists of 52 amino acids with an intramolecular disulfide bond, sharing slight homology with calcitonin gene-related peptide and amylin. Immunoreactive AM has subsequently been detected in plasma and a variety of tissues including blood vessels and lungs [13,47]. AM is metabolized by neutral endopeptidase protein in the kidney and by receptor binding in a variety of tissues. The half-life of AM is approximately 15 min. Earlier studies have shown that plasma AM level is increased in patients with hypertension [14] or heart failure [34]. Taking together its potent vasodilatory effect [19] and diuretic and natriuretic effects [21], AM may be involved in the regulation of the body fluid and thus in the cardiovascular homeostasis. We have shown that plasma AM level is elevated in patients with PPH, and that the plasma AM level increases in proportion to the severity of pulmonary hypertension [16]. It has been reported that there are abundant binding sites for AM in the lungs [37]. In fact, circulating AM is partially metabolized in the lungs [52]. These findings suggest that AM plays an important role in the regulation of pulmonary vascular tone and vascular remodeling. Earlier studies have shown that AM has a variety of biological effects, which are necessary for the treatment of pulmonary hypertension (Table 1). These actions of AM are mediated by calcitonin receptor-like receptor (CRLR) which functions as a selective AM receptor depending on the expression of the subtypes 2 and 3 of a family of receptor-activity-modifying proteins (RAMPs) [22]. AM acts through some signaling pathways: the cyclic adenosine 3′, 5′-monophosphate (cAMP), cyclic guanosine 3′, 5′-monophosphate (cGMP), phosphatidylinositol 3-kinase (PI3K)/Akt, and etc. These actions are induced by 0.01∼0.1 ug/(kg min) in vivo and by 10^{-10} to 10^{-7} M in vitro. This article will summarize the therapeutic potential of AM for the treatment of pulmonary hypertension.

2. Intravenous administration of AM

In vivo studies have shown that intravenously administered AM causes vasodilation, diuresis, and a positive inotropic effect in an experimental model of heart failure [40]. In humans, intravenous administration of AM decreases systemic and pulmonary vascular resistance and increases cardiac output in patients with congestive heart failure, together with slight increases in urine volume and urinary sodium excretion [31]. Endogenous AM production is enhanced in a variety of cardiovascular diseases through a compensatory mechanism [29]. Nonetheless, additional supplementation of AM has beneficial effects in these diseases [27]. These results suggest that endogenous AM level is not sufficient enough to improve deteriorated conditions in spite of the increased AM production.

Experimental studies have shown that intralobar arterial infusion of AM causes dose-related decreases in pulmonary vascular resistance under conditions of high pulmonary vascular tone [9,20,36]. The vasodilatory effect is mediated by cAMP-dependent and nitric oxide-dependent mechanisms [15,32]. Thus, AM is known to be one of the most potent endogenous vasodilators in the pulmonary vascular bed. However, little information is available regarding the hemodynamic effects of intravenously administered AM in patients with pulmonary hypertension. Accordingly, we examined the hemodynamic and hormonal responses to intravenous infusion of AM (0.05 μg/kg/min) or placebo, were examined in 13 patients with pulmonary arterial hypertension including PPH [28]. Because AM-induced hypotension

Table 1
Beneficial effects of adrenomedullin for the treatment of pulmonary hypertension

Biological activity	Second messenger or signal
1. Potent pulmonary vasodilation	cAMP, NO/cGMP, PI3K/Akt
2. Inhibition of endothelial cell apoptosis	PI3K/Akt
3. Inhibition of smooth muscle cell proliferation and migration	cAMP, Ca^{2+}
4. Positive inotropic effect	cAMP, protein kinase C, Ca^{2+} release or influx
5. Diuresis and natriuresis	NO/cGMP, cAMP
6. Suppression of aldosterone production	Ca^{2+}
7. Induction of angiogenesis	PI3K/Akt, MEK/ERK
8. Anti-inflammation	cAMP

cAMP: cyclic adenosine 3′, 5′-monophosphate, cGMP: cyclic guanosine 3′, 5′-monophosphate, PI3K: phosphatidylinositol 3-kinase, NO: nitric oxide, ERK: extracellular signal-regulated kinase, MEK: mitogen-activated protein ERK kinase.

may cause adverse effects in patients with pulmonary hypertension, we used a relatively low dose of AM. Intravenous infusion of AM increased plasma AM level in patients with pulmonary hypertension (15 ± 1 to 48 ± 8 fmol/ml, cf. 10 ± 1 fmol/ml in healthy subjects). Infusion of AM significantly decreased pulmonary vascular resistance by 32%. In addition, AM decreased systemic vascular resistance without inducing a marked hypotension. The hemodynamic effects of AM lasted at least 15 min after the end of infusion. These results suggest that AM has potent, relatively long-lasting pulmonary vasodilator activity in patients with pulmonary hypertension. We have shown that administered AM increases plasma cAMP, but not cGMP, in patients with pulmonary hypertension, in association with its hemodynamic effects. The increase in cAMP in smooth muscle cells by AM activates protein kinase A, resulting in the decrease in caicium content in smooth muscle cells. It is therefore possible that AM may relax vascular smooth muscle through a cAMP/protein kinase A-dependent mechanism. On the other hand, Nossaman et al. [36] have shown that AM regulates pulmonary vascular tone in rats through an endothelium-derived nitric oxide-dependent mechanism. Nishimatsu et al. [35] have shown that AM induces Akt activation in the endothelium via the Ca^{2+}/calmodulin-dependent pathway and that this is implicated in the production of nitric oxide, which in turn induced endothelium-dependent vasodilation. Because the vascular effects of AM are known to vary with species and vascular regions, further studies are necessary to elucidate the mechanisms responsible for pulmonary vasodilator activity of AM in humans.

Intravenous infusion of AM markedly increased cardiac index in patients with pulmonary hypertension [28], consistent with our previous results from left sided heart failure [31]. Considering the strong vasodilator activity of AM in the systemic and pulmonary vasculature, the significant decrease in cardiac afterload may be responsible for increased cardiac index with AM. On the other hand, a previous binding study has shown abundant, specific binding sites for AM in ventricular myocardium [37]. AM has been shown to increase cardiac cAMP [33], which is known to mediate the positive inotropic action of beta-adrenergic stimulants. Alternatively, AM has been shown to produce a positive inotropic action through cAMP-independent mechanisms [49]. These findings suggest that the increase in cardiac index may be attributable not only to a fall in cardiac afterload but also to the direct positive inotropic action of AM.

Infusion of AM significantly decreased plasma aldosterone, although there was no significant change in plasma renin activity. In vitro, AM has been shown to inhibit Ang II-induced secretion of aldosterone from dispersed rat adrenal zona glomerulosa cells [51]. Therefore, the inhibition of plasma aldosterone by AM was probably due to a direct effect on adrenal gland, as is the case for atrial natriuretic peptide [46].

It appears that a number of similarities in pharmacologic actions, i.e. vasodilatation, cardiac effect, and cAMP production, exist between AM and prostacyclin that is used for reducing pulmonary resistance in PPH. Unlike prostacyclin, however, AM has diuretic and natriuretic activities. AM inhibits inflammation and aldosterone production [7,51]. These biological effects may be the advantages of AM over prostacyclin in respect of therapeutic effectiveness. Exogenously administered AM at a pharmacologic level increased plasma cAMP in association with hemodynamic effects. Thus, additional administration of AM may be effective in patients with pulmonary hypertension.

3. Inhalation of AM

The goal of vasodilator therapy for patients with PPH is to reduce pulmonary vascular resistance without producing systemic hypotension, and improve quality of life and survival. We have shown that intravenous administration of AM markedly decreases pulmonary vascular resistance in patients with PPH [28]. Nevertheless, systemically administered AM decreases systemic arterial pressure, which may be harmful in treating patients with PPH. Recently, inhalation of aerosolized prostacyclin and its analogue, iloprost, has been shown to cause pulmonary vasodilation without systemic hypotension in patients with PPH [11,53]. In addition, inhalant application of vasodilators does not impair gas exchange because the ventilation-matched deposition of drug in the alveoli causes pulmonary vasodilation matched to ventilated areas. In clinical settings, inhalation therapy may be more simple, noninvasive, and comfortable than continuous intravenous infusion therapy. Thus, the purpose of this study was to investigate the effects of AM inhalation on hemodynamics and exercise capacity in patients with PPH.

Interestingly, Champion et al. [5] have shown that intratracheal gene transfer of calcitonin gene-related peptide (CGRP), a member of the same peptide family as AM, to bronchial epithelial cells attenuates chronic hypoxia-induced pulmonary hypertension in the mouse. These results raise the possibility that intratracheal delivery of a vasodilator peptide may be sufficient to alter pulmonary vascular function. In fact, inhalation of AM significantly decreased pulmonary vascular resistance in patients with pulmonary hypertension, whereas it did not alter systemic arterial pressure or systemic vascular resistance [26]. The ratio of pulmonary vascular resistance to systemic vascular resistance was significantly reduced by AM inhalation. These results suggest that inhaled AM improves hemodynamics with pulmonary selectivity. This is consistent with earlier findings that inhaled prostacyclin or its analogue, iloprost, acts transepithelially with pulmonary selectivity and improves pulmonary hypertension.

We examined the long-term effects of inhaled AM in monocrotaline (MCT)-induced pulmonary hypertension rats [30]. AM or saline was inhaled as an aerosol using an ultrasonic nebulizer, for 30 min, four times a day. Repeated

inhalation of AM for three weeks markedly decreased mean pulmonary arterial pressure and pulmonary vascular resistance in MCT rats without systemic hypotension. The potent, long-lasting pulmonary vasodilator effect of inhaled AM may contribute to the strong inhibition of the development of pulmonary hypertension. In addition, considering intermittent delivery of AM to the lungs, the chronic effects of inhaled AM appear to go beyond acute pulmonary vasodilation. Inhalation of AM inhibited an increase in the medial wall thickness of peripheral pulmonary arteries of MCT rats. In vitro studies have shown that AM inhibits the migration and proliferation of vascular smooth muscle cells [12,17]. Given the known potent vasoprotective effects of AM such as vasodilation and inhibition of smooth muscle cell migration and proliferation, it is interesting to speculate that AM trapped in the bronchial epithelium or alveoli leaks to the pulmonary arteries to maintain pulmonary vascular integrity in MCT rats. Importantly, Kaplan–Meier analysis demonstrated that the 6-week survival rate for MCT rats treated with aerosolized AM was significantly high (70%) as compared with 10% in those given saline [30]. Thus, treatment with aerosolized AM may be an alternative approach for severe pulmonary hypertension that is refractory to conventional therapy.

We have demonstrated that inhalation of AM has beneficial hemodynamic effects in animals and humans [26,30]. Recently, pulmonary delivery of a dry-powder insulin has been shown to improve glycemic control without adverse pulmonary effects [48]. Although further studies are necessary to maximize the efficiency and reproducibility of pulmonary AM delivery, combining AM inhalation therapy with other modalities that have a different mode of action may have beneficial effects in patients with PPH.

4. Cell-based AM gene transfer

The pulmonary endothelium plays an important role in the regulation of pulmonary vascular tone through the release of vasoactive substances such as nitric oxide and prostacyclin [6]. Dysfunction of the endothelium may play a role in the pathogenesis of pulmonary hypertension including PPH [4]. Thus, pulmonary endothelial cell may be a therapeutic target for the treatment of pulmonary hypertension. Recently, endothelial progenitor cells have been discovered in adult peripheral blood [2]. EPCs are mobilized from bone marrow into the peripheral blood in response to tissue ischemia or traumatic injury, migrate to sites of injured endothelium, and differentiate into mature endothelial cells in situ [8,18,50]. These findings raise the possibility that transplanted EPCs may serve not only as a tissue-engineering tool to reconstruct the pulmonary vasculature, but also as a vehicle for gene delivery to injured pulmonary endothelium. Thus, we investigated whether cell (EPCs)-based AM gene transfer ameliorates MCT-induced pulmonary hypertension in rats.

We obtained EPCs from cultured human umbilical cord blood mononuclear cells and constructed AM plasmid DNA. We used cationic gelatin to produce ionically linked DNA–gelatin complexes. Interestingly, EPCs phagocytosed plasmid DNA–gelatin complexes, which allowed nonviral, highly efficient gene transfer into EPCs [24]. Recently, intravenously administered hematopoietic cells have been shown to be attracted to sites of cerebral injury [39]. Intravenously injected EPCs accumulate in ischemic myocardium after acute myocardial infarction [18]. These findings suggest that progenitor cells have the capability to sense injured tissues. In fact, intravenously administered gene-modified EPCs were incorporated into pulmonary arterioles and capillaries in MCT rats and differentiated mature endothelial cells [25]. MCT injures endothelial cells of small arteries and capillaries in the lungs, resulting in pulmonary hypertension [44]. Taking these findings together, transplanted EPCs may circulate in the blood and attach to injured pulmonary endothelia in MCT rats. Thus, EPCs may serve not only as a vehicle for gene delivery to injured pulmonary endothelia, but also as a tissue-engineering tool in restoring intact pulmonary endothelium. Transplantation of EPCs without gene modification slightly, but significantly decreased pulmonary vascular resistance in MCT rats [25]. EPCs have been shown to express endothelial nitric oxide synthase and produce nitric oxide [24]. We showed that EPCs produce AM even when its gene is not transduced. These results suggest that vasodilator substances secreted from EPCs contribute to improvement in pulmonary hypertension. We also investigated whether transplantation of gene-modified EPCs causes further improvement in pulmonary hemodynamics and survival in MCT rats [25]. Interestingly, EPCs cultured with AM DNA–gelatin complexes markedly secreted AM protein for more than 2 weeks. These results suggest relatively long-lasting AM secretion from EPCs. The consequence of this synthesis in MCT rats was a marked decrease in mean pulmonary arterial pressure and pulmonary vascular resistance. Histological examination revealed that transplantation of AM-expressing EPCs inhibited an increase in medial wall thickness of pulmonary arteries. Expectedly, transplantation of AM-expressing EPCs caused significantly greater improvement in pulmonary hypertension and vascular remodeling than transplantation of EPCs alone. Given the known potent vasoprotective effects of AM such as vasodilation and inhibition of smooth muscle cell proliferation [12,17], it is interesting to speculate that AM secreted from EPCs may act not only as a circulating factor but also as a autocrine/paracrine factor in the regulation of pulmonary vascular tone and vascular remodeling in MCT rats. Importantly, a single transplantation of AM-expressed EPCs improved survival in MCT rats as compared with administration of EPCs alone or culture medium. These results suggest that ex vivo gene transfer into EPCs greatly enhances therapeutic effects of EPCs transplantation. Further studies are necessary to examine whether repeated administration of EPCs produces an even greater effect than single transplantation.

5. Summary

This article described the therapeutic potential of AM for the treatment of pulmonary hypertension. Baseline plasma AM is significantly higher in patients with pulmonary arterial hypertension. Nevertheless, exogenously administered AM at a pharmacologic level induces hemodynamic improvement. This suggests that an additional administration of AM may be effective in patients with pulmonary hypertension. We have demonstrated the effects of three types of AM delivery systems: intravenous administration of AM peptide, inhalation of AM peptide, and cell-based AM gene transfer. Further studies are necessary to examine which delivery system is the best in clinical settings. AM induces potent pulmonary vasodilation and has vasoprotective effects beyond vasodilation. Thus, AM is a promising endogenous peptide for the treatment of pulmonary arterial hypertension.

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