

DISCUSSION

The present study revealed for the first time that, in PH rats, the combination of an ET-A receptor antagonist and a PGI₂ analogue is more effective in inhibiting the progression of PH than single use of each drug alone. This conclusion is derived from the following results: (i) the combination of an ET-A receptor antagonist and a PGI₂ analogue inhibited the increase of RV systolic pressure and Pp/Ps to a greater degree than was obtained with either drug alone in PH rats, which data were also supported by the results examined by echocardiography; (ii) the combination of these drugs inhibited the medial wall thickening of pulmonary artery to a greater degree than was obtained with either drug alone; and (iii) the combination of these drugs inhibited the RV hypertrophy (increase in the ratios of RV weight to BW and RV weight to LV weight) due to high pressure load by PH to a greater degree than was obtained with either drug alone, which was accordant with the increased expression of β -MHC mRNA, a molecular marker for cardiac hypertrophy, in RV. From these findings, because single use of an ET-A receptor antagonist also ameliorated PH as well extent as a PGI₂ analogue, ET-1 pathway may play an important role in the progression of PH. A recent study showed that acute ET-A receptor blockade causes selective pulmonary vasodilation in human patients with PH due to chronic heart failure (29). At the present time, it is considered that the administration of PGI₂ is the most excellent drug for PH treatment in clinical field (1, 4, 11). Therefore, the findings in the present study will provide an important clinical implication that co-administration of an ET-A receptor antagonist and a PGI₂ analogue is superior in ameliorating PH to a PGI₂ analogue alone.

The important finding of this study is that co-administration of an ET-A receptor antagonist and a PGI₂ analogue was superior to the single use of each drug alone in ameliorating PH in rats. One of the beneficial effects of the combined therapy on PH is considered to be due to the additional effect of both drugs on vasorelaxation of the pulmonary arteries. BPS supplies PGI₂ to the pulmonary circulation, while TA-0201 blocks the binding of ET-1 to ET-A receptors. The signal transduction system differs between PGI₂ and ET-1; PGI₂ activates adenylate cyclase and increases cAMP which decreases intracellular Ca²⁺ (44, 45), while ET-1 activates phospholipase C

and diacylglycerol followed by an increase of inositol tri-phosphate and protein kinase C activation which increases intracellular Ca^{2+} (19, 46). Thus, the supplement of PGI₂ by BPS and blockade of ET-1 binding by TA-0201 may decrease intracellular Ca^{2+} and dilate the pulmonary arteries through additional mechanism of each compound. Furthermore, there is a possibility of interaction between PGI₂ and ET-1 in the induction of gene expression; for example, PGI₂ is reported to inhibit the expression of ET-1 gene in endothelial cells (19, 47) and the ET-1-induced DNA synthesis in vascular smooth muscle cells (48). Therefore, the combined use of two compounds for 19 days may inhibit the development of PH additionally through ablating the pharmacological action of ET-1.

One of the assumed mechanism of development of PH is that the impairment of vascular endothelial cell function of pulmonary vasculature (6). Rabinovitch et al. demonstrated by electron microscopy that characteristics of pulmonary vascular endothelial surface were altered in patients with PH, suggesting that there is an endothelial dysfunction in pulmonary vessels (49). Pulmonary vascular endothelial dysfunction leads to the imbalanced production of PGI₂ and TXA₂ (the decrease of PGI₂ and the increase of TXA₂), and this disproportion is considered to cause vascular spasm of pulmonary capillary vessels and microthrombus formation (6, 7, 50). The administration of BPS supplies PGI₂ to the pulmonary circulation in PH rats; therefore, vasospasm and generation of microthrombus may be inhibited. Thus, PH is ameliorated by the administration of BPS. This finding is consistent with a report that gene transfer of PGI₂ synthase ameliorates MCT-induced PH in rats (51).

The activation of ET-1 pathway in the pulmonary circulation is assumed to be involved in the progression of PH (22, 25). We previously reported that ET-1 immunoreactivity was very strong in the proliferated vascular smooth muscle cells and perivascular macrophages in the lungs of MCT-induced PH rats (30). These findings suggest that the increase of pulmonary arterial pressure is partly attributed to a potent vasoconstrictive action of ET-1. In addition, ET-1 has a potent proliferative effect on vascular smooth muscle cells (19, 20), which leads to the pulmonary vascular thickening and narrowing of the lumen. Thus, the another cause of the increase in the pulmonary vascular resistance and pulmonary arterial pressure may be partly attributed to the

vascular smooth muscle cell proliferation induced by ET-1. As the vascular smooth muscle cell proliferation is the result of chronic stimulation by endogenous substances, the pathophysiological involvement of ET-1 in PH may be through vascular smooth muscle cell proliferation as well as vasoconstriction. Therefore, it is considered that the chronic administration of TA-0201 suppresses the progression of PH through inhibiting the proliferation of pulmonary vascular smooth muscle cells in addition to inhibiting the vasoconstriction of the pulmonary vasculature.

In the present study, PH rats developed RV hypertrophy due to the high pressure load by PH. Both BPS and TA-0201 significantly and comparably suppressed the RV hypertrophy; moreover, the combination of these compounds inhibited the RV hypertrophy to the greatest degree among PH groups with various treatments. In myocardium, there are two subunits of MHC, α -MHC and β -MHC. Although the α -subunit is dominant in normal ventricle of rats, β -subunit becomes dominant when the load on the myocardium is increased, indicating that the expression of β -MHC is regarded as a molecular marker for cardiac hypertrophy (41, 42). The level of the expression of β -MHC mRNA showed the same pattern as the reduction of RV hypertrophy by these compounds. One of the mechanism of inhibiting the RV hypertrophy is considered to be due to the reduction of the pulmonary arterial pressure and pulmonary vascular resistance by these compounds. Because the mechanical load on cardiac myocytes induces proto-oncogene expression and cardiac hypertrophy (52), the reduction of the pulmonary arterial pressure and pulmonary vascular resistance by PGI₂ analogue and/or ET-A receptor antagonist attenuated the direct mechanical stress on cardiomyocytes in RV, which resulted in inhibition of RV hypertrophy. In addition, the following another mechanism of inhibiting the RV hypertrophy can be considered. ET-1 is produced by cardiac myocytes and has a potent cardiac hypertrophic effect both *in vitro* (21) and *in vivo* (53); we also have reported that pressure-overload increases the production of ET-1 in the heart and that the expression of ET-1 mRNA is elevated in the hypertrophied RV of PH rats (25, 31). Thus, another mechanism for the inhibition of RV hypertrophy development is suspected to be partly attributed to the interference by TA-0201 with the direct action of ET-1 on cardiac hypertrophy. Furthermore, as PGI₂ suppresses the induction of ET-1 gene (19, 47), the

administration of both compounds may inhibit the RV hypertrophy additionally through eliminating the pharmacological action of ET-1. Therefore, the administration of both compounds is considered to be a good combination for the treatment of RV hypertrophy and PH at the present time, because the excessive hypertrophy of RV in PH is regarded as a risk factor for right heart failure which brings high morbidity and mortality (1, 54).

As above-mentioned, the intravenous infusion of PGI₂ is an effective therapy for PH patients; however, it must be given continuously through a central intravenous line, and therefore, several complications may develop (1, 4, 13). Thus, the use of orally available drugs is expected and the combined use of an orally available ET-A receptor antagonist and an orally available PGI₂ analogue is considered to become an important strategy instead of continuous intravenous infusion of PGI₂ alone for the treatment of PH. Furthermore, initiating the combined therapy from the early stage of PH is likely to be preferable for inhibition of the remodeling of both pulmonary artery and RV.

Conclusions

This study suggests for the first time that the combined therapy with an ET-A receptor antagonist and a PGI₂ analogue inhibits the progression of PH and associated pulmonary vascular and RV remodeling to a greater degree than is obtained with either compound alone in MCT - induced PH rats. As these compounds are orally available, we can easily administer them to PH patients instead of the central intravenous infusion of PGI₂. Therefore, the present study provides an important clinical implication that the combination of an orally available ET-A receptor antagonist and an orally available PGI₂ analogue may become a new therapeutic strategy for treating PH.

Foot Note

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