Identification and characterization of novel antihypertensive peptides obtained from fermented milk

Hsiao-Ling Chen, Yu-Tang Tung, Gergeo Kuo, Chuan-Mu Chen

Department of Bioresources, Da-Yeh University, Changhua, Taiwan
Department of Life Sciences, Agricultural Biotechnology Center, National Chung Hsing University, Taichung, Taiwan
E-mail address: bellchen@mail.dyu.edu.tw (C.-M. Chen)

The fermented milk has antithrombotic, immunomodulatory, antihypertensive and cardiovascular effects. In the present study, the fermented milk and its derived peptide, 19AA, were investigated for the first time. Results demonstrated that the fermented milk had a strong antihypertensive activity in spontaneous hypertension rats (SHRs). Among two fractions derived from the fermented milk, Fraction A exhibited the best antihypertensive activity. Following reverse-phase high-performance liquid chromatography, a 19AA short peptide was further purified and identified from Fraction A. The synthetic peptide with a sequence of 19AA showed the strong antihypertensive activity from 1 to 10 h after oral administration of 1 mg of 19AA/kg of body weight, and the effect of systolic and diastolic blood pressure (SBP and DBP) decreasing was maximal at 44.0 ± 2.1 mmHg and 23.0 ± 2.9 mmHg, respectively, after oral administration. The present study revealed that 19AA showed excellent antihypertensive activity, and its activity is better than that of VPP tripeptide which has already included in functional foods. In conclusion, the 19AA fermented peptide is the bioactive ingredient with potential benefit in the prevention and treatment of hypertension or other associated disorders.


Purification different forms of extracellular superoxide dismutase and their effects on anti-hypertension through nitric oxide induction in spontaneous hypertension rats

Chuan-Mu Chen, Hsiao-Ling Chen, Ta-Yung Weng, Wei Chen, Yu-Tang Tung

Department of Life Sciences, National Chung Hsing University, Taichung, Taiwan
Department of Bioresources, Da-Yeh University, Changhua, Taiwan
Division of Pulmonary and Critical Care Medicine, Chia-Yi Christian Hospital, Chia-Yi, Taiwan
E-mail address: chchen1@dragon.nchu.edu.tw (C.-M. Chen)

Extracellular superoxide dismutase (EC-SOD), one member of SOD family, exists outside the cells in mammals. It catalyzes conversion of superoxide anion (\(O_2^-\)) into hydrogen peroxide (\(H_2O_2\)), destroying free radicals upon reactive oxygen species (ROS) production to relieve oxidative stress. In this study, a methylotrophic \textit{Pichia pastoris} system was used to produce a large-scale of recombinant human EC-SOD by yeast fermentation. After purified by fast protein liquid chromatography (FPLC), three different subtypes of EC-SOD were performed. Furthermore, the spontaneously hypertensive rat (SHR) was used to examine anti-hypertension efficacy among different EC-SOD isoforms through blood pressure measurement and NO release in blood after tail intravenous injection of EC-SOD. Results showed that different subtypes of EC-SOD share similar secondary structure but their activity diverse. After injection of high-dose EC-SOD into SHR, we observed obvious decrease of blood pressure and increase of blood nitric oxide (NO) immediately. After blocking the NO synthesis, EC-SOD lost its ability of blood pressure regulation. Our data provided that enormous antioxidant and antihypertensive activities of human EC-SOD were largely produced by yeast fermentation. It can be applied on the extent of health care to prevent hypertension and also cardiovascular diseases.


Counteracting effects of treprostinil and endothelin (ET-1) receptor antagonists (ETRAs) on endothelin-1, ETB receptor and ECE-1 levels in pulmonary smooth muscle cells (PASMCs) derived from patients with pulmonary arterial hypertension (PAH)

Jigisha Patel, Susan Hall, Lucie Clapp

Clinical Pharmacology Department, Division of Medicine, UK
E-mail addresses: rmhajip@ucl.ac.uk (J. Patel), rmhajip@ucl.ac.uk (S. Hall), rmhajip@ucl.ac.uk (L. Clapp)

Background: ET-1 levels rise in PAH, correlating with increased pulmonary vascular resistance and mortality. Lung endothelial ETB receptors promote ET-1 clearance, which is improved in PAH patients by epoprostenol (prostacyclin). Whether prostacyclin analogues behaviour similarly is unclear or if smooth muscle ETB receptors regulate ET-1 levels. Methods: Cultured human PASMCs from PAH patients were treated for 24 h with treprostinil, an ETRA or a combination. A chemiluminescent ELISA was used to measure ET-1 in the supernatant while ET-1 converting enzyme (ECE-1) and ETB receptors promote ET-1 clearance, which is improved in PAH patients by epoprostenol (prostacyclin). Whether prostacyclin analogues behaviour similarly is unclear or if smooth muscle ETB receptors regulate ET-1 levels. Results: Serum doubled ET-1 levels in HPSMCs, an effect abolished by treprostinil (10–100 nM). In contrast, the ETRAs (100 nM) bosentan and ambrisentan increased ET-1 levels by 100% while BQ788 (selective ETB antagonist) by 200%. Treprostinil in combination with these ETRAs failed to inhibit serum-induced ET-1 elevation. In contrast, BQ123 (selective ETA antagonist) did not affect the response to serum or treprostinil. ECE-1 protein levels were higher with BQ788 and bosentan compared to serum or treprostinil. Furthermore, ETB expression was down-regulated by treprostinil (10^(-9)M) significantly more than BQ788 (selective ETB antagonist) by 200%. Treprostinil in combination with these ETRAs failed to inhibit serum-induced ET-1 elevation. In contrast, BQ123 (selective ETA antagonist) did not affect the response to serum or treprostinil. ECE-1 protein levels were higher with BQ788 and bosentan compared to serum or treprostinil. Furthermore, ETB expression was down-regulated by treprostinil (10^(-9)M) significantly more than BQ788 (selective ETB antagonist) by 200%. Treprostinil in combination with these ETRAs failed to inhibit serum-induced ET-1 elevation. In contrast, BQ123 (selective ETA antagonist) did not affect the response to serum or treprostinil. ECE-1 protein levels were higher with BQ788 and bosentan compared to serum or treprostinil. Furthermore, ETB expression was down-regulated by treprostinil (10^(-9)M) significantly more than BQ788 (selective ETB antagonist) by 200%. Treprostinil in combination with these ETRAs failed to inhibit serum-induced ET-1 elevation. In contrast, BQ123 (selective ETA antagonist) did not affect the response to serum or treprostinil. ECE-1 protein levels were higher with BQ788 and bosentan compared to serum or treprostinil. Furthermore, ETB expression was down-regulated by treprostinil (10^(-9)M) significantly more than BQ788 (selective ETB antagonist) by 200%.
are counteracted by ETRAs targeting ETB but not ETA receptors. ETB receptors may also regulate ET-1 levels through changes in ECE-1 expression. We postulate that higher concentrations of treprostinil may be required to reach clinical efficacy in PAH when combined with non-specific ETRAs.


Potential involvement of functional tricuspid regurgitation in the diagnostic error to assess pulmonary arterial pressure by Doppler echocardiography
Saori Yamamoto, Yasuharu Matsumoto, Kotaro Nochioka, Masanobu Miura, Syunsuke Tatebe, Koichiro Sugimura, Tomoyuki Suzuki, Yoshihiro Fukimoto, Hiroaki Shimokawa

The Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan
E-mail address: yamamoto@cardio.med.tohoku.ac.jp (S. Yamamoto)

Background: Transthoracic Doppler echocardiography (DE) is useful for the screening of pulmonary hypertension (PH), which is often treated by endothelin antagonist, although recent studies have suggested that estimation of pulmonary artery pressure (PAP) by DE is frequently inaccurate. This study aimed to examine that functional tricuspid regurgitation (TR) with geometric alterations caused by right ventricular dilatation is involved in the diagnostic error of echocardiography. We conducted a retrospective cohort study of consecutive 127 patients (male, n = 58, mean age of 55 years) who received both echocardiography and right heart catheterization (RHC) during the 2-year period from November 2008 to October 2010. We defined PH as mean PAP > 25 mmHg at rest by RHC and “accurate estimated echocardiographic value” when it remained within 10 mmHg of the invasive measurement. Results: A total of 75 patients (59%) were diagnosed to have PH by RHC. When the patients were divided into 3 groups; accurate (n = 52), over-estimate (n = 63) and under-estimate (n = 12), the diagnosis of PH by RHC was 42% in accurate, 68% in over-estimate, and 83% in under-estimate groups (P = 0.004). In echocardiography, right ventricular dimension was significantly larger in over-estimate group (accurate, 30.0 ± 5.7 mm; over, 35.3 ± 8.6 mm; under, 32.8 ± 5.2 mm, P = 0.002), and the severity of TR was significantly worse in over-estimate group (P < 0.0001). Right atrium tended to be larger in both over-estimate and under-estimate groups than accurate group (accurate, 38.8 ± 5.7 mm; over, 42.6 ± 8.4 mm; under, 42.7 ± 6.2 mm, P = 0.073). Conclusions: Our results indicate that the accuracy of DE is not enough for PAP evaluation, particularly in patients with PH associated with increased TR grading and enlarged right heart dimension.


Detection of developing pulmonary vasculopathy with non-invasive cardiopulmonary exercise testing
Hiroki Kinoshita, Yoshihiro Dohi, Ryoji Sata, Yasuki Kihara

Department of Cardiovascular Medicine, University of Hiroshima, Hiroshima, Japan
E-mail address: yuyudohi@gmail.com (Y. Dohi)

Since the discovery of ET-1, over-expression of ET-1 has been demonstrated in patients with pulmonary arterial hypertension (PAH). In contrast to chronic thromboembolic pulmonary hypertension (CTEPH), patients with PAH have pulmonary vasculopathy (PV). PV leads to impaired dilatation of affected pulmonary vessels, impeding the increase of cardiac output (CO) and stroke volume (SV) during exercise. Peak O2 uptake shows CO, and peak O2–pulse shows SV during cardiopulmonary exercise testing (CPX). To investigate the increase of CO during exercise, we performed CPX in 12 patients with PAH and 7 patients with CTEPH. Predicted peak O2 uptake (45.5 ± 8.0 vs. 60.6 ± 13.4%, p < 0.01) and predicted peak O2–pulse (55.6 ± 7.6 vs. 69.1 ± 6.9%, p < 0.01) were significant higher in CTEPH than PAH. Diffusion capacity for carbon monoxide (%DLco: 40.3 ± 13.7 vs. 62.2 ± 13.9%, p < 0.01) was also significantly higher in CTEPH than PAH, however there was no correlation between %DLco and peak O2 uptake or peak O2–pulse. While, there was no difference in mean pulmonary arterial pressure (mPAP: 31 ± 6.8 vs. 30.1 ± 7.0 mmHg, n.s.), cardiac output (CO: 4.1 ± 0.6 vs. 4.4 ± 0.4 L/min, n.s.), and pulmonary vascular resistance (PVR: 5.2 ± 2.0 vs. 5.2 ± 2.8 wood units, n.s.) at rest. Our data indicate that, regardless of hemodynamic, both lower peak O2 uptake and peak O2–pulse show PV impeding the increase of CO during exercise. CPX can predict the onset of PAH by detection of PV in early stage.


Vascular endothelial growth factor (VEGF) and the control of endothelin-1 synthesis by human lung microvascular endothelial cells: A possible pathway for pathogenesis
Gregory Star, Michele Govinazzo, David Langleben

Center for Pulmonary Vascular Disease and Lady Davis Institute, Jewish General Hospital, Montreal, Canada
E-mail address: david.langleben@mcgill.ca (D. Langleben)

Introduction: Increased endothelin-1 (ET-1) is a hallmark of pulmonary arterial hypertension (PAH), and contributes to its pathogenesis. The factors controlling ET-1 in PAH are poorly understood. Vascular endothelial growth factor (VEGF) blockade results in PAH-like lesions in animal models, and has caused PAH in humans. The effects of VEGF on ET-1 production by human lung blood microvascular endothelial cells (HMVEC-LB) are unknown. Methods: We exposed HMVEC-LB (Lonza Inc.) in-vitro to human VEGF121 (40 ng/ml) in serum-free medium for 7 h, in the absence or presence of the VEGF receptor antagonist, SU5416 (Cayman Chemical, 3 and 10 μM). ET-1 production was measured in the supernatant. Phosphorylation of VEGF receptor 2 (VEGFR2) was measured by western blotting after exposure to VEGF ± SU5416 for 5 and 10 min. Results: VEGF effectively caused VEGFR2 phosphorylation, which was blocked by SU5416. VEGF decreased ET-1 production by 28%. In the absence of VEGF, SU5416 increased ET-1 production, by 16% at 10 μM, and SU5416 was able to completely abolish the VEGF effect on ET-1 production. Conclusion: VEGF may promote vascular health by decreasing ET-1 production in HMVEC-LB. Blockade of VEGF signalling by SU5416 increases ET-1 levels and may thereby contribute to the pathogenesis of pulmonary hypertension seen with VEGF blockade.


Effect of bosentan on exercise capacity in patients with pulmonary arterial hypertension or inoperable chronic thromboembolic pulmonary hypertension
Akihiro Hirashiki, Takahisa Kondo, Yoshihisa Nakano, Shiro Adachi, Shuzo Shimazu, Shinya Shimizu, Takahiro Okumura, Toyoaki Murohara

*Department of Advanced Medicine in Cardiopulmonary Disease, Nagoya University Graduate School of Medicine, Nagoya, Japan