Mitochondrial fission protein, dynamin-related protein 1, contributes to the promotion of hypertensive cardiac hypertrophy and fibrosis in Dahl-salt sensitive rats

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論文題目

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（ミトコンドリア分裂タンパクのダイナミン関連タンパク質１はダール食塩感受性ラットの高血圧性心肥大と心筋線維化形成に貢献する）

論文の内容の要旨

[Introduction]

Hypertension promotes left ventricular hypertrophy (LVH), which finally leads to cardiac dysfunction. Although aberrant mitochondrial dynamics is known to be a relevant contributor of pathogenesis in heart disease, little is known about the relationship between mitochondrial dynamics and LVH. We investigated the pathophysiological roles of Dynamin-related protein1 (Drp1, a mitochondrial fission protein) on the hypertensive LVH.

[Materials and Methods]

Animals, treatments and histological analysis:

This investigation conformed to the National Institute of Health Guide for the Care and Use of Laboratory Animals and was approved by the Hamamatsu University School of Medicine Animal Care and Use Committee. Salt-sensitive male Dahl rats were purchased at 6-week-old and started to feed with low salt (LS: 0.3% NaCl) or high salt (HS: 8% NaCl) chow at 7-week-old up to 15-week-old. Some rats were administrated with mdivi1 (an inhibitor of Drp1: 1 mg/kg of bodyweight by intraperitoneal injection) under HS chow feeding (M1). The isolated hearts were fixed with 4% paraformaldehyde, sliced in a short axis just below the mitral valve, and stained with hematoxylin-eosin or Azan. The left ventricular (LV) thickness and fibrosis were analyzed using ImageJ software.

Western blotting and measurement of reactive oxygen species (ROS):

The densitometry analysis of western blotting was performed using the ChemiDocTM system, and ROS was measured with OxiSelectTM assay kit using a plate reader.

Data analyses:

Data are presented as the means ± SEM and statistical analyses were performed using one-way analysis of variance (ANOVA) followed by the Bonferroni’s test. A level of $P < 0.05$ was considered statistically significant.

[Results]

A Drp1 inhibitor attenuated the hypertensive cardiac hypertrophy and cardiac fibrosis

In HS-group, the blood pressure, heart weight, and body weight and heart weight ratio (HW/BW) were higher than those of LS-group, while the laboratory examinations exhibited no significant alteration between HS- and LS-group. HS-groups exhibited significant LVH (wall thickness: 1.93 ± 0.05 mm, $P < 0.01$ vs. 1.37 ± 0.04 mm of LS-group) and myocyte hypertrophy (myocyte area: 1.6 ± 0.6 μm², $P < 0.01$ vs. 1.00 ± 0.3 μm² of LS-group), suggesting hypertensive cardiac hypertrophy. In addition, HS-group exhibited significant increase in fibrotic area than LS-group (fibrotic area: 15.5 ± 2.7%, $P < 0.01$ vs. 5.2 ± 0.8% of LS-group).

Because of critical contribution of Drp1 on the pathogenesis of cardiac hypertrophy, mdivi1 was treated with the HS-chow fed rats (M1-group). Although mdivi1 did not altered either the blood pressure or blood profile, mdivi1 reduced HW/BW, hypertension-induced LVH (wall thickness:1.42 ± 0.05 mm, $P < 0.01$ vs. HS-group), myocytes hypertrophy (myocyte area: 1.1 ± 0.05 μm², $P < 0.01$ vs. HS-group), and LV fibrotic area (3.3 ± 0.3%, $P < 0.01$ vs. HS-group) by hypertension, suggesting that mdivi1 suppressed the hypertension-induced LVH, cardiomyocyte hypertrophy, and cardiac fibrosis. In addition, mdivi1 significantly suppressed the hypertension-induced Drp1 expression, suggesting that Drp1 plays a role to promote the hypertension-induced LVH and cardiac fibrosis.

Mdivi1 suppressed ROS and hypertrophic signal molecules

The contribution of ROS was further investigated in our experimental condition. HS-group showed the increased ROS production and decreased superoxide dismutase 1 and 2 (endogenous ROS scavengers) expression, and mdivi1 attenuated those hypertension-induced reaction.

Calcineurin and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) are well-known cellular signaling molecules, which regulate the pathogenesis of cardiac hypertrophy with accompanying by the intracellular ROS production. HS-group exhibited significant increase in calcineurin and CaMKII, and mdivi1 suppressed those effects. Thus, excessive ROS production by increased Drp1 may activate calcineurin and CaMKII, which results in progression of cardiac hypertrophy in our experimental condition.

[Discussion]

In this study, using salt-sensitive Dahl rats, we investigated the crucial roles of aberrant mitochondrial dynamics on the pathogenesis of hypertension induced cardiac hypertrophy. The main findings of are as follows: (1) mdivi1 protected hearts from the hypertension-induced hypertrophy and fibrosis, (2) mdivi1 suppressed the hypertension-induced Drp1 and ROS production, and (3) mdivi1 suppressed the classical hypertrophic pathway like calcineurin and CaMKII. Although mdivi1 is not designed to suppress the Drp1 expression, but to inhibit an activity of GTPase (an active

site of Drp1), as we reported previously, mdivi1 may inhibit the mutual enhancement between ROS and Drp1 by hypertension.

[Conclusion]

The results led us to conclude the followings, (1) Drp1 is related with the hypertension-induced LVH, cardiac myocyte hypertrophy, and fibrosis, (2) mdivi1 can suppress them by inhibiting calcineurin and CaMKII through ROS reduction.