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研究課題名（和文）心房細動の発症におけるp53-miR34a-SIRT1フィードバック回路の役割

研究課題名（英文）The role of p53-miR34a-SIRT1 feedback loop in the development of atrial fibrillation

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研究成果の概要（和文）：心房細動の発症におけるp53-miR34a-SIRT1フィードバック回路の役割を明らかにした。
心房細動の発症には心房の電気的および器質的なリモデリングが関与するが、その分子生物学的機序は解明されていない。
我々は心房細動動物実験モデルを作成し、心房細動リモデリングにおけるp53-miR34a-SIRT1フィードバック回路の関与を検討した。

研究成果の概要（英文）：The role of p53-miR34a-SIRT1 feedback loop in the development of atrial fibrillation was elucidated.

研究分野：　循環器内科

キーワード：　心房細動

１．研究開始当初の背景

心房細動の発症には心房の電気的および器質的なリモデリングが関与するが、その分子生物学的機序は解明されていない。

２．研究の目的

心房細動リモデリングにおける p53-miR34a-SIRT1 フィードバック回路の関与を明らかにする。

３．研究の方法

心房細動動物実験モデルを作成し、心房細動リモデリングにおける p53-miR34a-SIRT1 フィードバック回路の関与を検討する

４．研究成果

心房細動の発症における p53-miR34a-SIRT1 フィードバック回路の役割を明らかにした。

心房細動の発症は心血管合併症のリスクの増加や生命予後の悪化に関連するが、抗不整脈薬による心房細動の停止および予防には大きな限界があり治療の有効性は未だに不十分である。治療成績向上のために発症機序のさらなる解明が不可欠である。心房細動の発症において心房の機能的および組織学的な変化“リモデリング”が重要な役割を持つ。発症機転の上流に位置する心房リモデリングを抑制し心房細動発症を予防する“アップストリーム治療”の効果に期待がもたれる。本研究は、イヌ疾患モデルを用いて心房細動の電気的および構造的基質の進展機序における、p53-miR34a-SIRT1 フィードバック回路の役割を、電気生理学的、組織学的、および分子生物学的に明らかにし、また p53-miR34a-SIRT1 フィードバック回路への介入による心房細動抑制の可能性を探索し、その成果によって心房細動の発症機序の解明および今後の心房細動治療の発展に寄与する事を目的とする。

当該年度（平成 28 年度）は前年度（平成 27 年度）動物実験モデルを用いた実験を施行し、従来の検討と矛盾しない実験結果を得た。すなわち高頻度の心房ペーシングにより、偽薬対照群では左心機能の低下とともに、心房有効不応期の短縮、心房内伝導時間の延長、そして心房細動持続時間の延長が確認されたが、アンジオテンシン-II 受容体拮抗薬投与群では、それらの変化が抑制され軽減された。さらに両群の心房組織を用いた分子生物学的検討を行い、偽薬対照群では心房組織における p53 の発現低下とトランスフォーミング増殖因子（TGF）-β1 の発現上昇がみられるのに対し、アンジオテンシン-II 受容体拮抗薬投与群ではそれらの変化が抑制される事を確認した。これらの結果は本研究の仮説である心房細動リモデリングの細胞内伝達機序における p53 の関与を示唆する所見である。

Effects of an angiotensin II receptor blocker, irbesartan (IRB), on the development of atrial fibrosis and atrial fibrillation (AF) were assessed in a canine model of atrial tachycardia remodeling (ATR) with left ventricular dysfunction, together with its possible association with involvement of p53. Atrial tachypacing (400 bpm for 4 weeks) was used to induce ATR in beagles treated with placebo (ATR-dogs, n = 6) or irbesartan (IRB-dogs, n = 5). Non-paced sham dogs served as control (Control-dogs, n = 4). ATR- and IRB-dogs developed tachycardia-induced left ventricular dysfunction. Atrial effective refractory period (AERP) shortened (83 ± 5 ms, $p < 0.05$), inter-atrial conduction time prolonged (72 ± 2 ms, $p < 0.05$), and AF duration increased (29 ± 5 s, $p < 0.05$ vs. baseline) after 4 weeks in ATR-dogs. ATR-dogs also had a larger area of atrial fibrous tissue (5.2 ± 0.5 %, $p < 0.05$ vs. Control). All these changes, except for AERP, were attenuated in IRB-dogs (92 ± 3 ms, 56 ± 3 ms, 9 ± 5 s, and 2.5 ± 0.7 %, respectively; $p < 0.05$ vs. ATR for each). In ATR-dogs, p53 expression in the left atrium decreased by 42 % compared with Control-dogs ($p < 0.05$); however, it was highly expressed in IRB-dogs (+89 % vs. ATR). Transforming growth factor (TGF)-β1 expression was enhanced in ATR-dogs ($p < 0.05$ vs. Control) but reduced in IRB-dogs ($p < 0.05$ vs. ATR). Irbesartan suppresses atrial fibrosis and AF development in a canine ATR model with left ventricular dysfunction in association with p53.

Echocardiographic characteristics and hemodynamic indices

Representative images of LV M-mode recordings from an ATR- (Fig. 1A) and an IRB-dog (Fig. 1B) at 4 weeks show development of LV dilatation and LV hypokinesis. Quantitative data show an increase in LV end-diastolic (Fig. 1C) and end-systolic (Fig. 1D) dimensions and a decrease in LV ejection fraction (Fig. 1E) with near maximal changes observed after 1 week of atrial tachypacing in both dog groups compared with the baseline data. There were no significant differences between ATR- and IRB-groups in these LV indices. LA area was also enlarged in both ATR- (Fig. 2A) and IRB-dogs (Fig. 2B)

compared to the baseline data. Quantitative data show a progressive increase in LA area in both dog groups without significant differences between the groups (Fig. 2C). Body weight and hemodynamic indices at 4 weeks are presented in the Table. No significant inter-group differences were observed in the body weight and hemodynamic indices at 4 weeks (Table).

Electrophysiological indices and AF promotion

AF inducibility (Fig. 3A) was enhanced and AF duration (Figs. 3B, C) was prolonged in ATR-dogs; however, these changes were significantly reduced in IRB-dogs (Figs. 3A-C). The changes in AERP over time at each basic cycle length are presented in Figs. 3D-F. AERP progressively shortened over a wide range of basic cycle lengths with flattening the rate adaptation of AERP. There was no significant difference between the two groups, indicating irbesartan had no effect on AERP changes. The shortest atrial pacing cycle length with 1:1 atrial capture was shortened (Fig. 4A) and P wave duration was unchanged (Fig. 4B). However, inter-atrial conduction time measured at 4 weeks was slightly longer in ATR-dogs than in IRB-dogs (Fig. 4C). Neither significant changes over time nor inter-group differences were observed in the ventricular rate during atrial tachypacing (Fig. 4D) and in the sinus rate after pacemaker deactivation (Fig. 4E).

Histopathological changes

Representative LA histological images from a Control-, an ATR- and an IRB-dog and quantitative data on the LA fibrous tissue area are shown in Fig. 5. Interstitial fibrous tissue was increased and bundles of myofibers were separated by thick layers of fibrous tissue in the LA of an ATR-dog (Fig. 5B). In contrast, no obvious interstitial fibrosis was observed in an IRB-dog (Fig. 5C). Fibrosis tissue area (Fig. 5D) was significantly increased in ATR-dogs, but not in IRB-dogs compared with Control-dogs.

Changes in p53 and TGF-β1 expression

The protein expression of p53 (Fig. 6A) in the LA tissue was reduced in ATR-dogs (0.6 ± 0.3) compared with Control-dogs (1.0 ± 0.1, $p<0.05$); however, it was upregulated in IRB-dogs (1.9 ± 0.6, $p<0.001$ vs. Control, and $p<0.01$ vs. ATR). In contrast, TGF-β1 (Fig. 6B) expression was enhanced in ATR-dogs (12.6 ± 0.1) compared with Control-dogs (1.0 ± 0.0, $p<0.01$) but it was reduced in IRB-dogs (2.0 ± 0.0, p=NS vs. Control, and $p<0.01$ vs. ATR). These data suggest that p53 is associated with angiotensin II-triggered and TGF-β1-mediated atrial fibrosis and AF development in the present canine model.

Discussion

The main findings of the present study are as follows. First, atrial tachypacing in dogs induced atrial remodeling, and irbesartan retarded the development of atrial structural remodeling and reduced AF inducibility. Second, p53 downregulation together with TGF-β1 upregulation was observed in the LA tissue from ATR-dogs. These changes were opposite in the direction in IRB-dogs, suggesting that p53 might be associated with angiotensin II-triggered and TGF-β1-mediated atrial fibrosis and AF development in the present model.

Potential mechanisms underlying the effects of irbesartan on atrial remodeling

In dogs with ventricular tachypacing-induced congestive heart failure, atrial angiotensin II concentration is increased [8] and more substantial fibrosis is observed in the LA than in the LV [4,21]. Atrial selective fibrosis was also found in mice that overexpressed TGF-β1 [22,23]. Angiotensin converting enzyme inhibitors and angiotensin II receptor blockers both suppressed atrial fibrosis and AF promotion in canine experiments and other situations [8,9,11,24]. In the present study, an angiotensin II receptor blocker, irbesartan, suppressed TGF-β1-overexpression, atrial fibrosis and AF induction, a finding consistent with prior observations [25,26]. In addition to the previous findings [25,26], the present study suggested that p53 could be associated with development of atrial fibrosis and AF in ATR-dogs with left ventricular dysfunction.

Role of p53 in tissue fibrosis

The tumor suppressor, p53, is one of the most frequently mutated genes in human cancer, and regulates many target genes in response to diverse stresses [27]. In experimental models of congestive heart failure, p53 expression in cardiomyocytes was upregulated, and caused apoptosis and cardiac dysfunction [28,29]. However, in fibroblasts, p53 suppressed TGF-β-regulated collagen gene expression [12]. In a mouse pressure-overload model and in in vitro cardiac fibroblasts, interruption of angiotensin II signaling

enhanced p53 protein expression, and suppressed fibroblast proliferation and collagen expression, whereas concomitant p53 knockdown restored cell proliferation and collagen expression [14,15]. These findings indicate that p53 may play a suppressive role in cardiac fibrosis [14]. In the present study, ATR was associated with p53 downregulation together with TGF-β1 overexpression and atrial fibrosis, a finding consistent with previous studies [12,14]. In addition, irbesartan suppressed TGF-β1 overexpression and atrial fibrosis along with upregulation of p53 expression, suggesting that p53 might have a suppressive role against atrial fibrosis and AF development in the present model.
Conclusion
In a canine model of AF induced by atrial tachypacing and tachycardia-induced LV dysfunction, an angiotensin II receptor blocker, irbesartan, is effective in suppressing atrial structural remodeling and AF development possibly in association with p53.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計　1　件）

Effect of irbesartan on development of atrial fibrosis and atrial fibrillation in a canine atrial tachycardia model with left ventricular dysfunction, association with p53. Kataoka N, Nishida K, Kinoshita K, Sakamoto T, Nakatani Y, Tsujino Y, Mizumaki K, Inoue H, Kinugawa K. Heart Vessels. 2016 Dec;31(12):2053-2060. Epub 2016 May 28.

〔学会発表〕（計　1　件）

Kataoka N, Nishida K, Kinoshita K, Sakamoto T, Nakatani Y, Mizumaki K, Inoue H. Involvement of the p53-miR34a-SIRT1 Positive Feedback-Loop Pathway in the Development of Atrial Fibrosis in a Canine Model of Atrial Fibrillation. 第 79 回日本循環器学会学術集会；2015 Apr 24-26；大阪.

〔図書〕（計　0　件）

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６．研究組織

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