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Differential effects of angiotensin II receptor blockers on Aβ generation

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HIGHLIGHTS

- Telmisartan markedly increased Aβ generation.
- Losartan, valsartan and candesartan did not increase Aβ generation.
- Olmesartan significantly increased Aβ42 generation.
- Telmisartan increased the Aβ generation through angiotensin type 1a receptor-PI3K pathway.

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ABSTRACT

Angiotensin II receptor blockers (ARBs) are widely prescribed for the medication of systemic hypertension and congestive heart failure. It has been reported that ARBs can reduce the risk for the onset of Alzheimer's disease (AD) and have beneficial effects on dementia. Neurotoxic amyloid β-protein (Aβ) is believed to play a causative role in the development of AD. However, whether ARBs regulate Aβ generation remains largely unknown. Here, we studied the effect of ARBs on Aβ generation and found that telmisartan significantly increased Aβ40 and Aβ42 generation, but decreased the Aβ42/Aβ40 ratio. However, losartan, valsartan and candesartan did not increase Aβ generation, while olmesartan significantly increased Aβ42 generation. We also found that telmisartan increased the Aβ generation through angiotensin type 1a receptor (AT1a) and the receptor-related phosphotidylinositide 3-kinases (PI3K) pathway. Our findings revealed the different effects of ARBs on Aβ generation and provide new evidence for the relationship between antihypertensive treatment and AD pathogenesis.

1. Introduction

Angiotensin II receptor blockers (ARBs) are used primarily for the treatment of hypertension where the patient has intolerance to angiotensin converting enzyme (ACE) inhibitor therapy since they are rarely associated with the persistent dry cough and/or angioedemia that limit ACE inhibitor therapy. The prevalence of hypertension increases to 51% in the group aged 60–74 years [1], and there is an almost 15-fold increase in the prevalence of dementia, predominantly Alzheimer's disease (AD), between the ages of 60 and 85 years [2]. AD is the most common form of dementia and is pathologically characterized by a reduction in brain volume, generally accompanied by brain shrinkage and cerebral amyloid plaques which are largely composed of neurotoxic amyloid β-protein (Aβ), which is generated from the cleavage of amyloid precursor protein (APP). Increasing evidence points to a link between hypertension and AD [3,4]. The epidemiological investigations have shown that AD rapidly progressed in elderly people who have a history of lifestyle-related diseases such as hypertension [5,6]. The clinical findings also indicate that cerebrovascular disease may play an important role in determining the presence and severity of the clinical symptoms of AD [7]. The incidence of AD is associated with the antihypertensive medications, including calcium-channel blockers [8], ACE inhibitors [9] and ARBs [10]. However, since the current

Abbreviations: ARBs, angiotensin II receptor blockers; AD, Azheimer's disease; Aβ, amyloid β-protein; AT1a, angiotensin type 1a receptor; ACE, angiotensin converting enzyme; MEFs, mouse embryonic fibroblasts; hAPP695, human 695-amino acid amyloid precursor protein; APP, amyloid precursor protein; ELISA, enzyme-linked immunosorbent assay; AT1, angiotensin II type 1 receptor; Ang II, angiotensin II; MAPK, mitogen activated protein kinase; PI3K, phosphotidylinositide 3-kinase; PKC, protein kinase C; AMPK, AMP-activated kinase; pAkt, phosphorylation of Akt; tAkt, total Akt.

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research on the association of ARBs and AD is limited to epidemiological research, there is no evidence concerning the direct effect of ARBs on Aβ generation. Here, we report the different effects of ARBs on Aβ generation among the ARBs examined. We found that telmisartan significantly increased Aβ40 and Aβ42 generation through the angiotensin type 1a receptor (AT1a) and the receptor related phosphotidylinositide 3-kinases (PI3K) pathway. Olmesartan significantly increased the Aβ42/Aβ40 ratio. However, losartan, valsartan and candesartan did not affect Aβ generation.

2. Materials and methods

2.1. Cell lines and cell culture

To generate *Agtr1a* deficient mouse embryonic fibroblasts (MEFs), we isolated MEFs from 13.5-day-old embryos of *Agtr1a* deficient mice (The Jackson Laboratory) with the C57BL/6 background, following the procedures as described previously [11]. All animal procedures were approved by the Iwate Medical University Committee for Animal Use. C57BL/6 MEFs and *Agtr1a* deficient MEFs were infected with human 695-amino acid amyloid precursor protein (hAPP695) cDNA by a retrovirus-mediated method according to published methods [12].

The two kinds of cells were cultured in DMEM (Wako Pure Chemical Industries) supplemented with 10% FBS (Sigma). Cells were maintained at 37 °C in an atmosphere of 5% CO_2 in a tissue culture incubator. The hAPP695 infected fibroblasts were passaged with the same cell concentration for each pathway inhibitor administration. After 1 h of incubation with the each inhibitor, the fibroblasts were treated with 20 μM telmisartan to examine which inhibitor reversed the increased Aβ generation by telmisartan.

2.2. Reagents

Telmisartan was purchased from Sigma and dissolved in DMSO. Losartan was purchased from Wako and dissolved in PBS. Valsartan, olmesartan and candesartan were purchased from TRC and were dissolved in DMSO. PD98059 and wortmannin were purchased from Cell Signaling. PD98059 was administered at the final concentration of 10 μM. Wortmannin was administered at the final concentration of 100 nM and 10 μM. Compound C and protein kinase C (PKC) inhibitor cocktail were purchased from Millipore. Compound C was administered at the final concentrations of 500 nM and the PKC inhibitor cocktail was administered at 5000 × dilution of the original solution. DAPT was purchased from Peptide Institute and was administered at the final concentration of 5 μM. PI3K activator is a 1732.8 Da peptide with the sequence KKHTDDGYMPMSPGVA and binds to the SH2 domain of the PI3K by the tyrosine phosphorylated version to activate the enzyme [13]. It was purchased from Santa Cruz Biotechnology. GW9662 was purchased from SIGMA and was administered at the final concentration of 10 μM.

2.3. Aβ measurement

Aβ40 and Aβ42 in conditioned media were measured using a sandwich enzyme-linked immunosorbent assay (ELISA) kit (Wako, Osaka, Japan), based on the manufacturer's instructions. Aβ40 and Aβ42 concentrations were normalized based on the amount of cell protein. All samples were measured in triplicate.

2.4. Cell lysate and cultured medium collection

Cells were lysed on ice in RIPA lysis buffer (50 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate and 0.1% sodium lauryl sulfate) containing a protease inhibitor mixture. The protein concentration of the collected supernatant was determined using a BCA protein assay kit (Thermo Fisher Scientific). Cell culture media was collected after centrifuging at 25,000 × *g* for 10 min at 4 °C and supplemented with a protease inhibitor.

2.5. Immunoblotting

APP fibroblasts were cultured to 70% confluence and starved overnight in serum-free medium prior to treatment. Starved cells were treated with 20 μM telmisartan for 5, 10, 15, 30 min and 1 h, and washed with 1 mM sodium orthovanadate before being lysed with 20 mM HEPES pH 7.0, 0.5% deoxycholic acid, 0.15 M NaCl, 0.1% SDS, 1% Nonidet P-40, 4 mM EDTA, 10 mM NaF, 10 mM $Na_4P_2O_7$, 2 mM sodium orthovanadate, containing a protease inhibitor cocktail (Roche). Wortmannin (100 nM) was administered prior to 20 μM telmisartan treatment. Equal amounts of protein from cell lysate were separated by SDS-PAGE and blotted onto polyvinylidene difluoride (PVDF) membranes (Immobilon). The membranes were incubated with the primary antibodies overnight at 4 °C. Appropriate peroxidase-conjugated secondary antibodies were applied and the membranes were visualized by SuperSignal Chemiluminesence (Thermo Scientific). Total Akt (tAkt) was detected on the same membrane after stripping the anti-pAkt antibody. The rabbit anti-Akt and anti-pAkt (Ser-473) antibodies were purchased from Cell Signaling. To examine the expression of AT1a, the brain of C57BL/6 mice and the APP fibroblasts were lysed in RIPA buffer and the same amount of proteins were separated by SDS-PAGE. The AT1a antibody using for immunoblotting was purchased from Bioss.

2.6. Statistical analyses

We compared group differences by one-way ANOVAs followed a post hoc Bonferroni test. Statistical analyses were carried out using GraphPad Prism 5. A *P*-value < 0.05 was considered to represent a significant difference. Graphs are expressed as means ± s.e.m.

3. Results

3.1. Telmisartan increased Aβ generation markedly, but significantly decreased the Aβ42/Aβ40 ratio

To determine whether ARBs affect Aβ generation, we infected C57BL/6J fibroblasts with hAPP695 cDNA to generate constant human APP overexpression fibroblasts (APP fibroblasts), and then treated them separately with telmisartan, losartan, valsartan, olmesartan or candesartan for 72 h. Telmisartan increased Aβ40 and Aβ42 generation markedly, about 6- and 3.2-fold (Fig. 1a and b), respectively, compared to the controls, while olmesartan increased Aβ40 generation about 2-fold at the concentration of 5 μM (Fig. 1a) and Aβ42 generation about 3.2-fold (Fig. 1b) at the concentration of 10 μM. However, losartan, valsartan and candesartan did not show any clear increase in the Aβ generation (Fig. 1a and b). The Aβ42/Aβ40 ratio in serum increased in familial AD patients and is considered as a causal factor of AD [14]. Among the ARBs examined, telmisartan significantly decreased the Aβ42/Aβ40 ratio and had the lowest Aβ42/Aβ40 ratio, whereas, olmesartan had the highest Aβ42/Aβ40 ratio which was significantly higher than that of telmisartan (Fig. 1c). Losartan, valsartan and candesartan did not show any clear effect on Aβ42/Aβ40 ratio (Fig. 1c).

3.2. Telmisartan significantly increased Aβ generation via AT1a

Telmisartan has the highest affinity for angiotensin II type 1 receptor (AT1) among the examined ARBs [15]. Telmisartan also