의학 석사 학위 논문

저산소증 상태에서 알츠하이머병 사이브리드 세포의 HIF-1α와 BACE 발현에 대한 스타틴의 효과

Statin effect on the expression of HIF-1α and BACE in Alzheimer's disease cybrid cells under hypoxia

2013년 2월

서울대학교 대학원

의학과 뇌신경과학 전공

정 진 헌

저산소증 상태에서 알츠하이머병 사이브리드 세포의 HIF-1α와 BACE 발현에 대한 스타틴의 효과

Statin effect on the expression of HIF-1α and BACE in Alzheimer's disease cybrid cells under hypoxia

지도교수 한문구

이 논문을 의학석사 학위논문으로 제출함.

2012년 10월

서울대학교 대학원

의학과 뇌신경과학 전공

정 진 헌

정진헌의 의학석사 학위논문을 인준함.

2012년 1월

위원장 김상윤 (인)

부위원장 한문구 (인)

위원 권오기 (인)

학위논문 원문제공 서비스에 대한 동의서

본인의 학위논문에 대하여 서울대학교가 아래와 같이 학위논문 저작물을 제공하는 것에 동의합니다.

1. 동의사항

① 본인의 논문을 보존이나 인터넷 등을 통한 온라인 서비스 목적으로 복제할 경우 저작물의 내용을 변경하지 않는 범위 내에서의 복제를 허용합니다.

② 본인의 논문을 디지털화하여 인터넷 등 정보통신망을 통한 논문의 일부 또는 전부의 복제·배포 및 전송 시 무료로 제공하는 것에 동의합니다.

2. 개인(저작자)의 의무

본 논문의 저작권을 타인에게 양도하거나 또는 출판을 허락하는 등 동의 내용을 변경하고자 할 때는 소속대학(원)에 공개의 유보 또는 해지를 즉시 통보하겠습니다.

3. 서울대학교의 의무

①서울대학교는 본 논문을 외부에 제공할 경우 저작권 보호장치(DRM)를 사용하여야 합니다.

②서울대학교는 본 논문에 대한 공개의 유보나 해지 신청 시 즉시 처리해야 합니다.

논문제목: 저산소증 상태에서 알츠하이머병 사이브리드 세포의 HIF-1α와 BACE 발현에 대한 스타틴의 효과

학위구분 : 석사 ▣· 박사 □

학 과 : 의학과 뇌신경과학 전공

학 번 : 2011-21904

연 락 처 :

저 작 자 : 정 진 헌 (인)

제 출 일 : 2013 년 2 월 1 일

서울대학교총장 귀하

Abstract

Statin effect on the expression of HIF-1α and BACE in Alzheimer's disease cybrid cells under hypoxia

Jin-Heon Jeong

Graduate Program of Neuroscience

Department of Medicine

The Graduate School

Seoul National University

Background: Alzheimer's disease (AD) is a progressive neurodegenerative disorder affecting memory function and higher cognitive function. Vascular risk factors have an established association with AD, and brain ischemia facilitates the pathogenesis of AD by accumulating beta amyloid (Aβ). Hypoxia increases the expression of β-site amyloid precursor protein cleaving enzyme (BACE) through overexpression of hypoxia inducible factor 1α (HIF-1α), resulting in accumulation of Aβ. Statins (HMG-CoA reductase inhibitor) have protective effects against hypoxia, and long-term statin treatment is known to decrease the risk of developing AD.

Methods and Results: In this study, we used mitochondrial transgenic neuronal cell (cybrid) models to investigate the changes of intracellular HIF-1α and BACE levels with administration of statins under hypoxia. Sporadic AD (SAD) cybrids and age-matched control (CTL) cybrids were incubated under hypoxia (93%

N2/5% CO2/2% air), and then 1 μM or 10 μM simvastatin was administrated. Intracellular HIF-1α and BACE levels were measured using Western-blot analysis. Under hypoxia, CTL and SAD cybrids showed reduced viability over time, and the intracellular expression of HIF-1α and BACE was increased. After administration of 1 μM simvastatin, the intracellular levels of HIF-1α and BACE were decreased in SAD cybrids. With 10 μM simvastatin, the intracellular expression of HIF-1α and BACE was increased in both SAD and CTL cybrids.

Conclusion: A low-dose statin reduces the expression of HIF-1α and BACE under hypoxia in SAD cybrids. In contrast, a high-dose statin aggravates the expression of HIF-1α and BACE in both SAD and CTL cybrids. These results suggest that low-dose statins are more beneficial than high-dose statins in the prevention of Aβ production.

Key words: Statin, Alzheimer's disease, hypoxia.

Student Number: 2011-21904

List of Figures

Contents

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder affecting memory and higher cognitive function in the elderly population. The disease is characterized pathologically by the formation of brain senile plaques composed of aggregated forms of β-amyloid (Aβ)(1). Vascular risk factors such as hypertension and diabetes mellitus have an established association with AD, and over 30% of AD patients show evidence of cerebral infarcts(2, 3). Brain ischemia is recognized to contribute to the pathogenesis of AD(2, 3), and a molecular link between hypoxia and Aβ production is well established. Hypoxia increase β-site amyloid precursor protein cleaving enzyme (BACE) gene expression through overexpression of hypoxia inducible factor 1α (HIF-1α), resulting in increased β-secretase activity and Aβ production(4-6).

Statins (HMG-CoA reductase inhibitor) reduce the incidence of ischemic stroke and improve functional outcome after ischemic stroke(7). Statins upregulate nitric oxide synthase, which plays an important role in preserving blood flow and limiting neurological loss(8). Experimental data point to the neuroprotective properties of statins in acute cerebral ischemia(8). Beyond their original role in lowering cholesterol, statins are used for the management of neurodegenerative disorders such as vascular dementia and AD(9).

Epidemiologists found a decreased risk of AD in people taking statins, and patients on long-term statin treatment show decreased risk of developing AD up to

70%(10). Several experimental studies demonstrate that statins reduce the production of Aβ, and cellular cholesterol levels may be central to statin-mediated reduction in Aβ production(11). Low cellular cholesterol levels favor the α-secretase pathway and decrease Aβ secretion(12). Statins also alter HIF-1α related gene expression(13); HIF-1α plays an essential role in cellular and systemic response to hypoxia(14).

We investigated how intracellular HIF-1α and BACE levels change when statins are administered under hypoxic conditions in sporadic Alzheimer's disease cybrids(15, 16).

Materials and Methods

Cell culture experiments

Mitochondrial transgenic neuronal cells (cybrids) of sporadic AD (SAD) were used to investigate the effect of statins on HIF-1α and BACE expression under hypoxic conditions. In this technique, mitochondria and mitochondrial DNA (mtDNA) were transferred to culturable cells depleted of endogenous mtDNA. We transferred mitochondria and mitochondrial genes from the platelets of alive AD patients to the SH-SY5Y cell line which is deficient in mitochondria. To create cybrids, SH-SY5Y cells lacking mtDNA were repopulated with mitochondria containing platelet mtDNA, which were received from volunteer patients or their age-matched disease free controls (CTL). The resulting cell lines differed only in the source of mtDNA that repopulated the cells, but otherwise had identical nuclear genetic and environmental backgrounds, allowing the in vitro elucidation of mitochondrial genomic differences.

Studies using this technique have demonstrated that SAD cybrids have increased intracellular and/or extacellular Aβ levels that mediate increased apoptotic neuronal death(17). SAD cybrids also showed increased accumulation of oxidative stress markers such as trans-4-hydroxy-2-nonenal adducts(18).

In vitro hypoxia

Cell cultures were maintained in Dulbecco's Modified Eagle's Medium (DMEM)

supplemented with 10% fetal bovine serum (FBS), 100 U of penicillin and 0.1 mg/ml streptomycin at 37°C under 5% CO2/95% air. The cells were maintained in a plate at 37°C in 95% air/5% CO2 until reaching 70% confluence. After starving with DMEM containing 0.2% FBS for 24 hours, the cultures were placed in normoxic or in hypoxic conditions with 1 μM or 10 μM simvastatin (Chong Kun Dang Pharmaceutical Co., South Korea) throughout the time course of the experiments (0 to 12 hours).

All hypoxic experiments were performed with the cells incubated in a humidified hypoxic chamber. In order to induce hypoxia, the cultures were incubated in 93% N2/5% CO2/2% air at 37°C.

Cell viability assay

Cell viability was determined by MTT (3,[4,5-dimethylthiazol-2-yl]2,5-diphenyl tetrazolium bromide) assay. A stock solution of MTT (5 mg/ml in phosphate-buffered saline, pH 7.4) was freshly prepared and the cells were incubated for 4 hours at a final concentration of 1 mg/ml. The samples on each plate were read on an ELISA reader with a reference wavelength of 570 nm. The results were expressed as a percentage of absorbance at 490 nm directly proportional to the number of living cells following experimental hypoxia.

HIF and BACE immunoassay

For immunoblot analysis, the cells cultured on the 100 mm plate were washed with 4°C phosphate-buffered saline (PBS) and collected. They were homogenized in lysis buffer (100 mmol/L NaCL, 10 mmol/L Tris (pH 7.5), 1 mmol/L EDTA) to which protease inhibitors (1 mM phenylmethylsulfonyl fluoride) were freshly added. Protein concentrations were determined using the Bradford method (Bio-Rad, Richmond, CA). Protein extracts (40 ug) were separated by 6~10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane. They were blocked in 5% nonfat skim milk in TBS (0.15 M NaCL, 25 mM Tris-HCL, 25 mM Tris) for 2 hours, and then incubated at a 1:500 dilution overnight at 4℃ with anti-BACE (rabbit polyclonal antibodies, Millipore corporation) or anti-HIF-1α (mouse monoclonal antibodies, BD bioscience) antibodies. After washing 3 times in TBST (TBS + 0.5% Tween-20), the membrane was incubated with secondary antibody (anti-rabbit or anti-mouse) for 1 hour at room temperature. Immunoreactive bands were detected by enhanced chemiluminescence with Kodak film. All experiments were repeated three times.

Results

Hypoxia increased cell death

Initially, we analyzed the effect of simvastatin on cell viability under hypoxia. During hypoxia (2% air condition) for 12h, SAD and CTL cells showed reduced viability over time (Fig. 1). Between 0h and 3h, viability was reduced to 50% of the control level, and after 12h, 70% of the cells were dead. After administration of 1 μM simvastatin, there was no difference between cell survival in both SAD and CTL cybrids (Fig. 1a, 1b). Administration of 10 μM simvastatin also did not affect cell survival in both SAD and CTL cybrids (Fig. 1c, 1d).

Low-dose simvastatin decreased the intracellular expression of HIF-1α and BACE in SAD cybrids

In order to determine the effect of statin on HIF-1α mediated BACE expression, an immunoassay was performed. Hypoxia increased the intracellular expression of HIF-1α and BACE in both CTL and SAD cybrids (Fig. 2, 3). In SAD cybrids, the HIF-1α level was increased at 3h and 6h, and then began to decrease at 12h (Fig. 2a). During hypoxia for 12h, the BACE level was gradually increased in SAD cybrids (Fig. 2b). Under hypoxia, HIF-1α expression was rapidly increased for the first 6h, whereas BACE expression was slowly increased and continued for 12h. After administration of 1 μM simvastatin, the intracellular HIF-1α and BACE levels were decreased in SAD cybrids (Fig. 2a, 2b). Densiometric analysis showed

that the HIF-1α level was significantly decreased by 70% (3h), 40% (6h), and 40% (12h) with 1 μM simvastatin (*P<0.05) (Fig. 2a). The BACE level was significantly decreased at 12h (40%), but there was little change at 3h (<10%) and 6h (10%) (Fig. 2b). In SAD cybrids, 1 μM simvastatin reduced the expression of HIF-1α and BACE after hypoxia. This reduction of HIF-1α expression was prominent at 3h, and the reduction of BACE expression was pronounced at 12h.

In CTL cybirds, hypoxia increased the intracellular expression of HIF-1α and BACE, but administration of 1 μM simvastatin did not significantly affect the intracellular expression of HIF-1α and BACE (Fig. 3).

High-dose simvastatin increased the intracellular expression of HIF-1α and BACE in both SAD and CTL cybrids

Hypoxia increased the intracellular expression of HIF-1α and BACE in both CTL and SAD cybrids. Contrary to 1 μM simvastatin, administration of 10 μM simvastatin increased the expression of intracellular HIF-1α and BACE (Fig. 4, 5). Densiometric analysis showed that the HIF-1α level was increased by 110% (3h), 20% (6h) and 20% (12h) in SAD cybrids (Fig. 4a). In CTL cybrids, the HIF-1α level was increased by 110% (3h), 110% (6h) and 130% (12h) (Fig. 4b). With 10 μM simvastatin, the expression of HIF-1α was significantly increased at 3h in SAD cybrids and at 3h, 6h and 12h in CTL cybrids. Densiometric analysis results revealed that the BACE level was increased by 60% (3h), 10% (6hr) and 10% (6hr) in SAD cybrids (Fig. 5a). In CTL cybrids, the BACE level was increased by 30%

(3h), 20% (6h) and 20% (12h) (Fig. 5b). With 10 μM simvastatin, the expression of BACE was significantly increased at 3h in both SAD and CTL cybrids. Under hypoxia, 10 μM simvastatin augmented the expression of HIF-1α and BACE in both SAD and CTL cybrids.

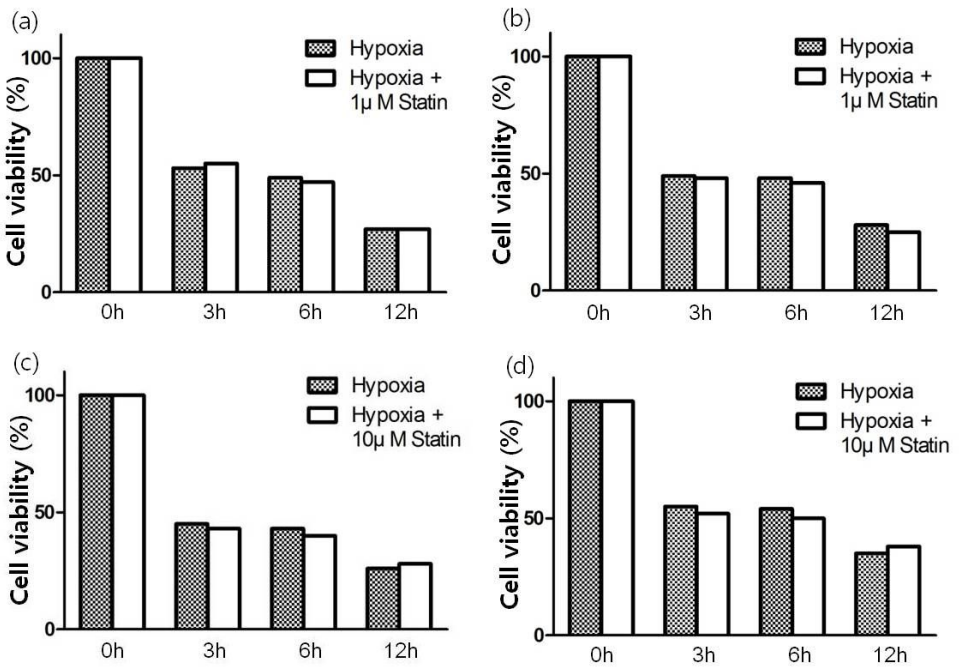

Figure1. Simvastatin did not affect cell viability in both SAD and CTL cybrids. Cells were incubated in a humidified hypoxic chamber (93% N2/5% CO2/2% air) with 1 μM or 10 μM simvastatin. Cell viability was determined by MTT assay. (a) The effect of 1 μM simvastatin on cell viability in SAD cybrids. (b) The effect of 1 μM simvastatin on cell viability in CTL cybrids. (c) The effect of 10 μM simvastatin on cell viability in SAD cybrids. (d) The effect of 10 μM simvastatin on cell viability in CTL cybrids. ; X axis denotes hypoxia duration, Y axis denotes percentage of viability comparing to the 0 time point. All experiments were repeated three times.

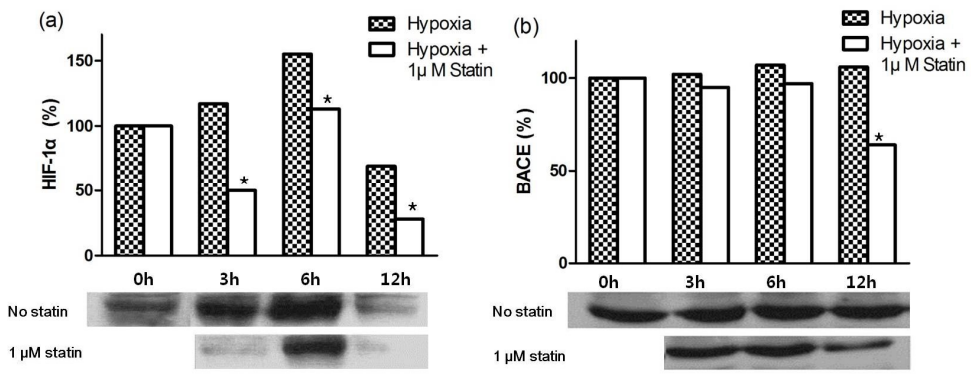

Figure2. Low-dose simvastatin inhibited the intracellular expression of HIF-1α and BACE in SAD cybrids. Cells were incubated with 0 and 1 μM simvastatin under hypoxia. Intracellular HIF-1α and BACE levels were measured using Western-blot analysis. (a) The effect of 1 μM simvastatin on HIF-1α expression in SAD cybrids. (b) The effect of 1 μM simvastatin on BACE expression in SAD cybrids ; X axis denotes hypoxia duration, Y axis denotes percentage immunoassay comparing to the 0 time point. All experiments were repeated three times.

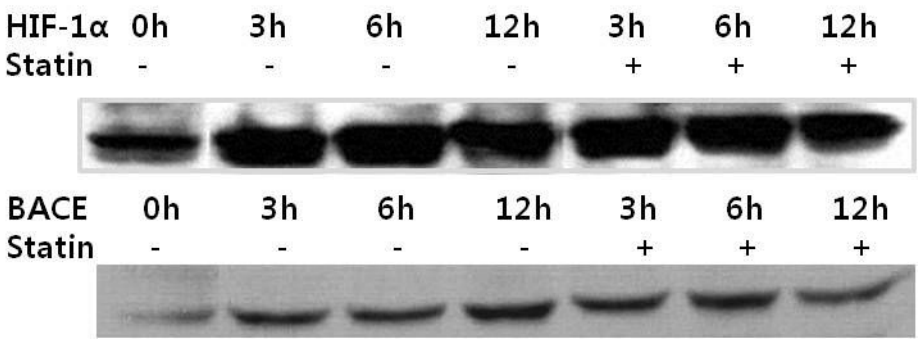

Figure3. Low-dose simvastatin did not affect the intracellular expression of HIF-1α and BACE in CTL cybrids. CTL cybrids were incubated with 0 and 1 μM simvastatin under hypoxia. Intracellular HIF-1α and BACE levels were measured using Western-blot analysis. All experiments were repeated three times.

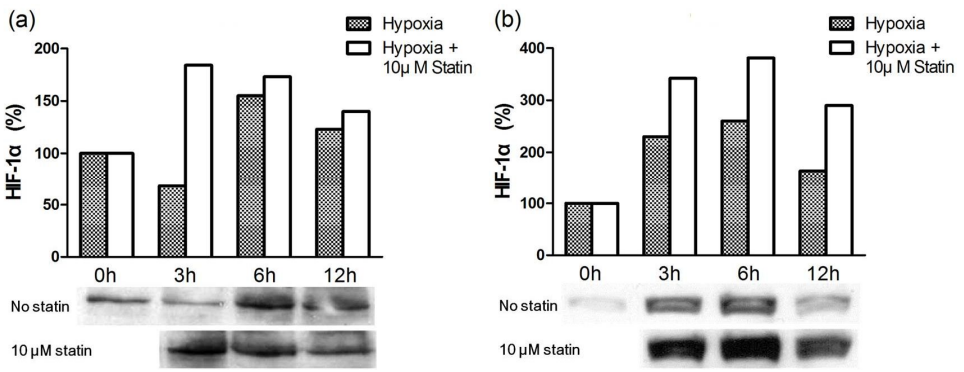

Figure 4. High-dose simvastatin increased the intracellular HIF-1α level. Cells were incubated with 0 and 10 μM simvastatin under hypoxia. Intracellular HIF-1α level was measured using Western-blot analysis. (a) The effect of 10 μM simvastatin on HIF-1α expression in SAD cybrids. (b) The effect of 10 μM simvastatin on HIF-1α expression in CTL cybrids. ; X axis denotes hypoxia duration, Y axis denotes percentage immunoassay comparing to the 0 time point. All experiments were repeated three times.

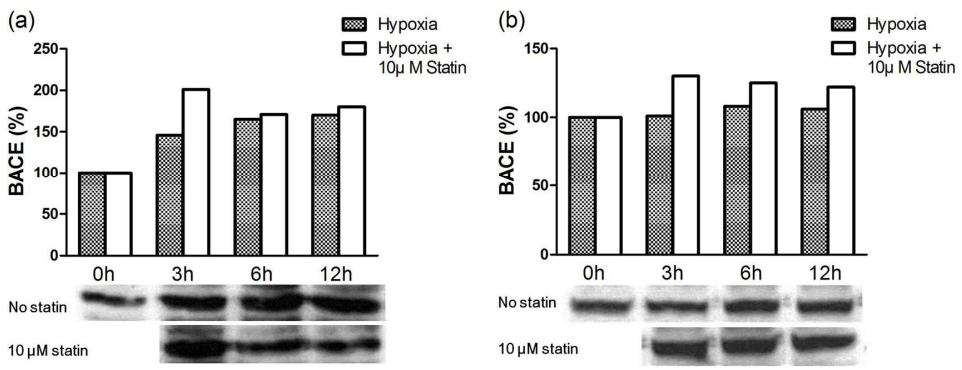

Figure 5. High-dose simvastatin increased the intracellular BACE level. Cells were incubated with 0 and 10 μM simvastatin under hypoxia. Intracellular BACE level was measured using Western-blot analysis. (a) The effect of 10 μM simvastatin on BACE expression in SAD cybrids. (b) The effect of 10 μM simvastatin on BACE expression in CTL cybrids. ; X axis denotes hypoxia duration, Y axis denotes percentage immunoassay comparing to the 0 time point. All experiments were repeated three times.

Discussion

This study investigated the changes of cellular HIF-1α and BACE levels with administration of statin under hypoxic conditions in Alzheimer's disease cybrid cells. In SAD cybrids, the intracellular HIF-1α and BACE levels decreased with a low-dose statin. Contrary to our expectation, a high-dose statin increased the expression of HIF-1α and BACE. Aβ is derived from the β-amyloid precursor protein by sequential proteolytic cleavage from β-secretage and γ-secretage(4-6). Hypoxia increase the gene transcription of BACE through overexpression of HIF-1α(5). A low-dose statin reduced the HIF-1α mediated BACE production against hypoxic injury in SAD cybrids. These results suggest that low-dose statins are more beneficial than high-doses for the prevention of Aβ production.

There are other studies that show dose-dependent effects of statin. One study demonstrated that atorvastatin dose-dependently affects endothelial cell migration and angiogenesis(19). Low-dose statins promote migration of mature endothelial cells and progenitor cells that contribute to vasculogenesis(19). However, high-dose statin block angiogenesis and migration by inducing endothelial cell apoptosis(19). In cortical neuronal cells, a low-dose simvastatin (100 nmol) protects neurons from cytotoxcity by enhancing Bcl-2 mRNA expression(20). Pre-incubation with a low-dose simvastatin reduces cell death by toxicity of Aβ peptide in both cortical and cerebellar neurons(21). If these in vitro experiments correctly reflects pathophysiological events that take place in the human brain, then low-

dose statins may have more therapeutic benefits. Further studies on the dose-dependent effects of statin are warranted.

Statins inhibit both cholesterol and isoprenoid synthesis. High-dose statins inhibit cholesterol synthesis, and low cellular cholesterol levels decreases Aβ secretion(12, 13). Low-dose statins inhibit isoprenoid biosynthesis(22, 23), and inhibition of β-secretase dimerization by low isoprenoid reduces Aβ production(24, 25). Our experiments also showed reduced BACE levels with 1 μM of simvastatin. However 10 μM of simvastatin increase BACE levels, and it could not be explained by the cholesterol and isoprenoid mediated mechanism. Under hypoxic conditions, HIF-1α mediated BACE expression become more prominent, and BACE level is more affected by HIF-1α expression. A previous one study showed that simvastatin attenuated HIF-1α expression in vascular smooth muscle cells(24). Statins modulate the DNA binding activity of HIF-1α(9, 24), but little is known about the interaction of statins with HIF-1α.

Alzheimer's disease patients are susceptible to chronic hypoxia. As we predicted, SAD cybrids are also susceptible to chronic hypoxia. Disrupted perfusion is present in the early phases of AD(2), and consequently, a reduction of oxygen delivery to brain tissue promotes mitochondrial dysfunction and apoptosis(17). We used a cybrid cell model to evaluate mitochondrial dysfunction of cells of AD patients under hypoxic conditions. Mitochondrial dysfunction to oxidative stress is intimately involved with AD pathophysiology. The mitochondrial electron chain acts as an oxygen sensor, releasing ROS in response to hypoxia, thereby promoting

oxidative stress, leading to cell death(25). Mitochondrial dysfunction is observed in platelets and lymphocytes of AD patients and their postmortem brain tissue(26).

Several studies showed that statin actions on Aβ production is mediated by BACE(23, 27, 28). Although we did not directly measure the Aβ level, intracellular Aβ production is estimated by measuring the intracellular BACE level. In vitro and in vivo studies showed that overexpression of BACE sequentially elevates Aβ production(29, 30). This up-regulation of BACE and hypoxic stress is thought to have pathogenic relevance to neurodegeneration and dementia.

In conclusion, the present study showed that a low-dose statin reduced the expression of HIF-1α and BACE under hypoxia in SAD cybrids. In contrast, a high-dose statin aggravated the expression of HIF-1α and BACE in both SAD and CTL cybrids. These results suggest that low-dose statins are more beneficial than high-dose statins in the prevention of Aβ production.

References

1. Braak H, Braak E. Evolution of the neuropathology of Alzheimer's disease. Acta Neurol Scand Suppl. 1996;165:3-12.

2. Royall DR. Alzheimer disease as a vascular disorder: nosological evidence. Stroke. 2002;33(9):2147-8.

3. Koistinaho M, Koistinaho J. Interactions between Alzheimer's disease and cerebral ischemia--focus on inflammation. Brain Res Brain Res Rev. 2005;48(2):240-50.

4. Sun X, He G, Qing H, Zhou W, Dobie F, Cai F, et al. Hypoxia facilitates Alzheimer's disease pathogenesis by up-regulating BACE1 gene expression. Proc Natl Acad Sci U S A. 2006;103(49):18727-32.

5. Zhang X, Zhou K, Wang R, Cui J, Lipton SA, Liao FF, et al. Hypoxia-inducible factor 1alpha (HIF-1alpha)-mediated hypoxia increases BACE1 expression and beta-amyloid generation. J Biol Chem. 2007;282(15):10873-80.

6. Guglielmotto M, Aragno M, Autelli R, Giliberto L, Novo E, Colombatto S, et al. The up-regulation of BACE1 mediated by hypoxia and ischemic injury: role of oxidative stress and HIF1alpha. J Neurochem. 2009;108(4):1045-56.

7. Lakhan SE, Bagchi S, Hofer M. Statins and clinical outcome of acute ischemic stroke: a systematic review. Int Arch Med. 2010;3:22.

8. Stepien K, Tomaszewski M, Czuczwar SJ. Neuroprotective properties of statins. Pharmacol Rep. 2005;57(5):561-9.

9. Wang Q, Yan J, Chen X, Li J, Yang Y, Weng J, et al. Statins: multiple

neuroprotective mechanisms in neurodegenerative diseases. Exp Neurol. 2011;230(1):27-34.

10. Jick H, Zornberg GL, Jick SS, Seshadri S, Drachman DA. Statins and the risk of dementia. Lancet. 2000;356(9242):1627-31.

11. Sidera C, Parsons R, Austen B. The regulation of beta-secretase by cholesterol and statins in Alzheimer's disease. J Neurol Sci. 2005;229-230:269-73.

12. Cole SL, Grudzien A, Manhart IO, Kelly BL, Oakley H, Vassar R. Statins cause intracellular accumulation of amyloid precursor protein, beta-secretase-cleaved fragments, and amyloid beta-peptide via an isoprenoid-dependent mechanism. J Biol Chem. 2005;280(19):18755-70.

13. Dichtl W, Dulak J, Frick M, Alber HF, Schwarzacher SP, Ares MP, et al. HMG-CoA reductase inhibitors regulate inflammatory transcription factors in human endothelial and vascular smooth muscle cells. Arterioscler Thromb Vasc Biol. 2003;23(1):58-63.

14. Ogunshola OO, Antoniou X. Contribution of hypoxia to Alzheimer's disease: is HIF-1alpha a mediator of neurodegeneration? Cell Mol Life Sci. 2009;66(22):3555-63.

15. King MP, Attardi G. Human cells lacking mtDNA: repopulation with exogenous mitochondria by complementation. Science. 1989;246(4929):500-3.

16. Swerdlow RH, Parks JK, Miller SW, Tuttle JB, Trimmer PA, Sheehan JP, et al. Origin and functional consequences of the complex I defect in Parkinson's disease. Ann Neurol. 1996;40(4):663-71.

17. Onyango IG, Tuttle JB, Bennett JP, Jr. Altered intracellular signaling and reduced viability of Alzheimer's disease neuronal cybrids is reproduced by beta-amyloid peptide acting through receptor for advanced glycation end products (RAGE). Mol Cell Neurosci. 2005;29(2):333-43.

18. Onyango IG, Bennett JP, Jr., Tuttle JB. Endogenous oxidative stress in sporadic Alzheimer's disease neuronal cybrids reduces viability by increasing apoptosis through pro-death signaling pathways and is mimicked by oxidant exposure of control cybrids. Neurobiol Dis. 2005;19(1-2):312-22.

19. Urbich C, Dernbach E, Zeiher AM, Dimmeler S. Double-edged role of statins in angiogenesis signaling. Circ Res. 2002;90(6):737-44.

20. Johnson-Anuna LN, Eckert GP, Franke C, Igbavboa U, Muller WE, Wood WG. Simvastatin protects neurons from cytotoxicity by up-regulating Bcl-2 mRNA and protein. J Neurochem. 2007;101(1):77-86.

21. Bate C, Williams A. Squalestatin protects neurons and reduces the activation of cytoplasmic phospholipase A2 by Abeta(1-42). Neuropharmacology. 2007;53(2):222-31.

22. Bellosta S, Via D, Canavesi M, Pfister P, Fumagalli R, Paoletti R, et al. HMG-CoA reductase inhibitors reduce MMP-9 secretion by macrophages. Arterioscler Thromb Vasc Biol. 1998;18(11):1671-8.

23. Frears ER, Stephens DJ, Walters CE, Davies H, Austen BM. The role of cholesterol in the biosynthesis of beta-amyloid. Neuroreport. 1999;10(8):1699-705.

24. Wilson SH, Herrmann J, Lerman LO, Holmes DR, Jr., Napoli C, Ritman

EL, et al. Simvastatin preserves the structure of coronary adventitial vasa vasorum in experimental hypercholesterolemia independent of lipid lowering. Circulation. 2002;105(4):415-8.

25. Chandel NS, Maltepe E, Goldwasser E, Mathieu CE, Simon MC, Schumacker PT. Mitochondrial reactive oxygen species trigger hypoxia-induced transcription. Proc Natl Acad Sci U S A. 1998;95(20):11715-20.

26. Chaturvedi RK, Beal MF. Mitochondrial approaches for neuroprotection. Ann N Y Acad Sci. 2008;1147:395-412.

27. Parsons RB, Price GC, Farrant JK, Subramaniam D, Adeagbo-Sheikh J, Austen BM. Statins inhibit the dimerization of beta-secretase via both isoprenoid- and cholesterol-mediated mechanisms. Biochem J. 2006;399(2):205-14.

28. Parsons RB, Farrant JK, Price GC, Subramaniam D, Austen BM. Regulation of the lipidation of beta-secretase by statins. Biochem Soc Trans. 2007;35(Pt 3):577-82.

29. Tamagno E, Bardini P, Obbili A, Vitali A, Borghi R, Zaccheo D, et al. Oxidative stress increases expression and activity of BACE in NT2 neurons. Neurobiol Dis. 2002;10(3):279-88.

30. Kao SC, Krichevsky AM, Kosik KS, Tsai LH. BACE1 suppression by RNA interference in primary cortical neurons. J Biol Chem. 2004;279(3):1942-9.

요약 (국문 초록)

배경: 알츠하이머병은 기억력과 고위 인지기능의 저하를 특징으로 하는 신경 퇴행성 질환이다. 알츠하이머병과 여러 혈관성 위험 인자와의 연관성이 잘 알려져 있으며, 뇌허혈은 아밀로이드 베타를 증가시키고 이를 통해 알츠하이머병의 진행을 촉진시킨다. 산소 결핍에 대한 반응으로 hypoxia inducible factor 1α (HIF-1α)의 발현이 증가되고, 이는 β-site amyloid precursor protein cleaving enzyme (BACE)의 증가를 통해 아밀로이드 베타를 증가시킨다. 스타틴은 저산소증에 대해 보호작용이 있는 것으로 알려져 있으며, 역학연구에서 장기간 스타틴을 복용하면 알츠하이머병의 위험도를 감소시킨다는 보고가 있었다.

방법과 결과: 이 연구에서는 알츠하이머병 환자와 정상 대조군의 미토콘드리아를 신경아세포종 세포에 이식한 사이브리드 세포 모델을 사용하여, 저산소증 상태에서 스타틴 투여에 따른 세포 내의 HIF-1α와 BACE의 변화를 알아보고자 하였다. 사이브리드 세포는 1 μM 혹은 10 μM의 스타틴 투여 후 저산소증 (2% 산소) 상황에서 배양되었다. 세포내의 HIF-1α와 BACE의 변화는 웨스턴-블럿 검사를 통해 측정하였다. 저산소증 상황에서 사이브리드 세포의 수는 감소되었고, 세포내의 HIF-1α와 BACE의 발현은 증가하였다. 알츠하이머병 사이브리드 세포에서 1 μM 스타틴을 투여하였을 때 투여 전과 비교하여 세포 내의 HIF-1α와

BACE의 발현이 감소하였다. 반면에 10 μM 스타틴을 투여하였을 때는 알츠하이머병과 정상 대조군 사이브리드 세포에서 투여 전과 비교하여 세포 내의 HIF-1α와 BACE의 발현이 증가시켰다.

결론: 저용량의 스타틴은 알츠하이머병 사이브리드 세포에서 HIF-1α와 BACE의 발현을 감소시켰다. 반면에 고용량의 스타틴은 알츠하이머병과 정상 대조군 사이브리드 세포에서 오히려 HIF-1α와 BACE의 발현을 증가시켰다. 이런 결과는 알츠하이머병 사이브리드 세포에서 1 μM 스타틴의 투여가 저산소증에 의한 아밀로이드 베타의 증가에 대해 효과적일 수 있다는 것을 의미한다.

주요어 : 스타틴, 알츠하이머병, 저산소증

학번 : 2011-21904