Review Article

Anti-Aging Medicine in Cardiovascular Disease

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Introduction

“A man is as old as his arteries.” This statement was made by William Osler in 1898 and is still currently used by many researchers and clinicians as a witty remark. It constitutes a core concept of Anti-Aging. About 100 years has passed since he made this remark, and as we live in an age of plentiful food we now really understand how significant his statement is.

The use of vascular age values calculated from measurement of vascular functions has gradually been increasing in routine medical examination and consultation. From now on, it will be increasingly important to link these data to practical strategies for the purpose of improving longevity coupled with health, *i.e.*, for prevention of diseases and reduction of events related to ischemic diseases. For such linking, it is considered essential to accumulate both basic and clinical scientific evidence for vascular senescence.

In this symposium, specialists in the concerned fields on both the basic and clinical sides have gathered to provide state-of-the-art knowledge under the theme of Anti-Aging Medicine in Cardiovascular Disease and regarding the question, “What is vascular senescence?”, conducting analyses of association with other diseases primarily involving blood vessels and the relationships between Anti-Aging measures, the renin-angiotensin system, and other relevant topics. We hope that the audience will pick up interesting findings that may be implemented in their daily research and acquire knowledge that will be helpful in their medical practices.

Cognitive Function and Angiotensin Receptor Subtypes

Cognitive Function and the Renin-Angiotensin System

Stroke ranks third among all causes of death in Japanese people. Silent multiple cerebral infarction noted in the elderly leads to cognitive function impairment and is a major cause of cerebrovascular dementia. In stroke management practices, primary prevention by improving lifestyle-related habits is of the utmost importance, and in particular, adequate control of blood pressure is most effective in preventing initial onset and recurrence of stroke. Blood pressure control using antihypertensive agents is the best “preventive therapy.” Various antihypertensive agents have recently attracted attention because of their additional benefits beyond antihypertensive effects. For stroke, results of large-scale clinical studies have demonstrated that antihypertensive agents which inhibit the renin-angiotensin system (RAS), such as angiotensin II type 1 (AT1) receptor blockers (ARBs) and angiotensin converting enzyme inhibitors (ACEIs), are effective in preventing the onset of cerebrovascular disorders, and also in suppressing impairment of cognitive function.

Using angiotensin II type 2 (AT2) receptor knockout (AT2KO) mice, we prepared an experimental model of middle cerebral artery occlusion (MCAO), evaluated the size of focal infarct lesions, and reported an increase in size of focal ischemic infarctions in AT2KO mice compared with wild-type mice. The infarct size in wild-type mice given an ARB was reduced, with size in AT2KO mice after valsartan administration reduced to a smaller extent. This finding indicated involvement of signals from AT2 receptors ¹⁾. The increased infarct size might have been related to a lack of signals via AT2 receptors, resulting in decreased blood flow and increased oxidative stress in the areas surrounding the focal infarct lesion ¹⁾.

Expression of AT2 receptors is abundant primarily in the embryonic development stage ²⁾ and decreases after birth, leading to a greater expression of AT1 than of AT2 receptors (*i.e.* reversal of AT1/AT2 ratio). In adult tissues, expression of AT1 receptors is

predominant and AT2 receptors are rarely expressed under normal conditions. Expression of AT2 receptors in the brain is usually limited to specific regions such as the cerebellum or olivary nucleus³⁾. One study indicated that expression of AT2 receptors increased following organ disorders such as heart failure, renal failure, and brain damage⁴⁾. In addition, AT2 receptors are powerfully expressed in the regions responsible for regulating learning or exercise function and it has been reported that ARBs or ACEIs enhance cognitive function⁵⁾. In light of these findings, signals from AT2 receptors may be deeply involved in organ development and cell differentiation, and in repair and regeneration of nerve injuries.

MMS2 and AT2 Receptor Signals

In the present investigation, we focused on methyl methanesulfonate sensitive 2 (MMS2), a neural differentiation related factor. MMS2 is a ubiquitin-conjugating enzyme (E2) variant reported to bind to the E2 Ubc-13, contributing to DNA repair⁶⁾. Expression of MMS2 is enhanced in rat fetal brain, indicating its involvement in the development and differentiation of brain⁷⁾. We investigated how activation of signals from AT2 receptors affects MMS2 expression and found that the expression is intensified. We further evaluated how signals from AT2 receptors are involved in and influence nerve protection and neural differentiation, giving consideration to the association with MMS2. Neurospheres usually do not stick to the bottom of petri dishes; however, stimulation with Ang II increased the number of neurosphere cells attached to the petri dish bottom and these attached neurospheres differentiated into nerve cell-like cellular morphologies. Results showed increased expression of β -tubulin and MAP2, markers for differentiation of nerve cells, whereas no change was noted in expression of GFAP, a marker for differentiation of glial cells. These phenomena were intensified by addition of valsartan, an ARB. Further, none of these effects were noted when the AT2 receptor antagonist PD 123319 was used, even in the presence of Ang II. It is therefore considered that signals via AT2 receptors may promote differentiation of nerve stem cells and play a substantial role in determining their development into nerve cells.

Next, we investigated expression of MMS2, which is reported to be a factor for neural differentiation because its expression increases in the stages of rat brain development and growth. Stimulation with Ang II intensified MMS2 expression and this was considered due to signals mediated by AT2 receptors. To evaluate the functions of MMS2 in detail, we introduced siRNA into a neurosphere to knock down MMS2 and observed subsequent changes. The neurosphere into which siRNA of MMS2 had been introduced changed and collapsed as a condensed mass of cells coming apart. The collapsed cells were attached and did not differentiate into nerve cells, possibly even undergoing apoptosis, given the cell morphology observed. The above-described findings indicate that stimulation by AT2 receptors may enhance MMS2 expression to protect nerve cells and play an important role in differentiation.

Next, we performed the shuttle avoidance test to evaluate cognitive function and learning capacity in AT2KO mice. Comparison of wild-type and AT2KO mice that did not substantially differ in exercise function revealed a remarkable decrease in avoidance rate after MCAO treatment in AT2KO mice. Interestingly, the avoidance rate in these mice was also lowered before infarction. It is therefore considered that the lack of AT2 receptors may have some influence on neuronal maturation and neuroarchitecture as described above, and also on cognitive function and learning capacity. Treatment with the ARB valsartan

suppressed the reduction of post-infarction avoidance rate. Given that valsartan does not affect blood pressure at the dosage level used in our investigation, we may conclude that the suppression caused by valsartan is an effect beyond its hypertensive action. We also investigated MMS2 expression in brain and found that intracerebral expression of MMS2 in infarct regions after cerebral infarction was increased in wild-type mice but not in AT2KO mice. Given that expression of AT2 receptors increases following organ disorders such as brain damage, MMS2 expression may also be regulated via AT2 receptors, which increase under some disease conditions. Accordingly, it is considered that signals via AT2 receptors may be involved, via MMS2, in inhibition of post-infarction impairment of cognitive function. In our steadily aging society, prevention of disturbance of higher brain functions such as memory, learning, and cognition is becoming an extremely important and challenging issue. For cognitive function impairment, the effects of blood pressure alone on the impairment have been shown and the Systolic Hypertension in Europe (Syst-Eur) Study⁸⁾ has demonstrated that blood pressure reduction suppresses the onset of dementia. Large-scale clinical studies such as the Perindopril Protection Against Recurrent Stroke Study (PROGRESS) have proven that lowering blood pressure will prevent decline of cognitive function⁹⁾. It is therefore suggested that the effects of antihypertensive agents on cognitive function are related to hypertension. However, it was reported that ARBs significantly improved cognitive function scores according to the Sandoz Clinical Assessment Geriatric (SCAG) scale and the Mini Mental State Examination (MMSE) when compared with thiazide diuretics. There is evidence indicating that ARBs and ACEIs are effective in preventing the onset of cerebrovascular disorders when compared with the other antihypertensive agents in spite of equivalent antihypertensive effect among these agents, and that they contribute to secondary prevention. In light of these findings, ARBs may not only act directly on the nerves but may also be effective in preventing vascular dementia.

Calcification of Blood Vessels and Underlying Mechanisms for Osteoporosis

Estrogen and Bone/Vascular Age

It is well known that bone and vascular ages advance rapidly in postmenopausal women. The appropriateness of hormone replacement therapy has been controversial since a study of a hormone replacement therapy with estrogen plus progestin was partially discontinued due to increased risks of cardiovascular events and other relevant diseases. More specifically, in the Women's Health Initiative (WHI), 16,608 postmenopausal women aged 50-79 years received conjugated estrogen at 0.625 mg in combination with progesterone at 2.5 mg and were followed up for a mean of 5.2 years. Results showed that risk of cardiovascular disease events in these women was 1.29 times higher (95% confidence interval (CI), 1.02-1.63) and risk of breast cancer was 1.26 times higher (95% CI, 1.00-1.59), whereas risk of fracture was reduced by a factor of 0.66 (95% CI, 0.45-0.98)¹⁰⁾. Another Women's Health Initiative (WHI) study involved 10,739 postmenopausal women with hysterectomy aged 50-79 years who received conjugated estrogen at 0.625 mg and were followed up for a mean of 6.8 years. Risks of cardiovascular disease events and breast cancer in these women did not increase as previously noted, but rather decreased by a factor of 0.91 (95% CI: 0.75-1.12) and

0.77 (95% CI: 0.59-1.01), respectively; and a risk of fracture decreased as expected by a factor of 0.66 (95% CI: 0.41-0.91)¹¹⁾. There is no doubt that hormone replacement therapy is extremely effective for treating osteoporosis. When considering the involvement of hormone replacement therapy in the onset of cardiovascular events, however, we must keep in mind that consequences of the therapy vary depending on when, how, and on whom it is performed. In addition, from the viewpoint of mechanism, so-called vascular functions such as arteriosclerosis need to be distinguished from risks of thrombosis.

New guidelines for hormone replacement therapy have recently been published stating that, "When properly used, hormone replacement therapy is useful for promoting and maintaining QOL (Quality of Life) of postmenopausal women, although it may sometimes bring about harmful effects. In particular, when a hormone replacement therapy is newly started in women aged 60 years or older, the risks may outweigh the benefits, and therefore the benefits and risks of the therapy should be thoroughly investigated." More recently, a study has shown that transdermal estrogen differs from oral estrogen in efficacy and adverse reactions. For thromboembolism, a multicenter case-control study comparing oral and transdermal preparations showed that the risk of thromboembolism in treated individuals was 3.5 times higher (95% CI: 1.8-6.8) than in non-treated individuals for oral formulation, a value which greatly differed from the value of 0.9 (95% CI: 0.5-1.6) for transdermal preparation¹²⁾. From now on, selection of a route of administration should be considered an important factor in administering hormone replacement therapy.

Classification of Blood Vessels and Bone Metabolism

As age-related vascular changes, arteriosclerosis and vascular calcification progress with age. It has been recently clarified that osteogenesis molecules localized in the blood vessels are involved in this vascular calcification. We focused on the receptor activator for nuclear factor kappa B ligand/osteoclast differentiation factor (RANKL/ODF) system as a molecular mechanism common to disease condition establishment in both osteoporosis and vascular calcification.

The RANKL system plays a central role in disease condition formation of osteoporosis. The molecule is primarily produced by osteoblasts, and activates osteoclasts, thereby leading to osteoporosis. Two receptors exist for the RANK ligand: RANK and a decoy receptor osteoprotegerin (OPG), which regulate the activation of osteoclasts by RANKL. In OPG-deficient mice, RANKL was excessively stimulated and a phenotype presenting simultaneous osteoporosis and vascular calcification was observed. These RANKL-related molecules are also expressed in vascular cells; indeed, an investigation in mice demonstrated an increase in expression of these molecules along with vascular calcification. We found that addition of RANKL to cultured vascular endothelial cells increased bone morphogenetic protein (BMP)-2, an osteogenesis molecule, and decreased matrix Gla protein (MGP), an inhibitory factor for calcification. Addition of RANKL to an experimental system in which cultured vascular smooth muscle cells were made to undergo bone differentiation in a medium inducing osteoblast differentiation promoted bone differentiation. These findings obtained by the addition of RANKL indicated a possibility that RANKL may accelerate calcification of vascular smooth muscle. This RANKL-promoted bone differentiation was inhibited by estrogen treatment. We also have established a new mouse model that concurrently develops vascular calcification and osteoporosis, and are currently investigating the effects of RANKL system on vascular calcification.

Lifestyle-Related Diseases from the Viewpoint of Anti-Aging

Cellular Level Senescence and Vascular Senescence

In aged blood vessels, compliance is decreased, inflammation is accelerated, and the anti-thrombosis effect of the endothelium is reduced. For example, in aged vascular endothelia, endothelial nitric oxide synthase (eNOS) activity is decreased¹³⁾. The reduction in vascular functions increases risks of arteriosclerotic cardiovascular diseases with aging. In fact, it has been confirmed that arteriosclerotic lesions in human contain cells that resemble aging cells in morphology. The senescence-associated β -galactosidase (SA- β -gal) assay makes use of the increased activity of β -gal in pH6 in aged cells. A study has confirmed that SA- β -gal-positive endothelial cells may be noted on the surface of coronary plaques but not in non-arteriosclerotic lesions of the internal thoracic artery or other relevant arteries¹⁴⁾.

The telomere hypothesis is considered important as a mechanism of cellular senescence. Telomeres exist at the ends of chromosome and function as a substrate in protection and repair of the chromosomes. Telomeres are shortened every time a cell divides, and extreme shortening of the telomere induces p53/p21 signal pathway-dependent cellular senescence. Telomeres form loops called t-loops to prevent the chromosomal end from being recognized as a DNA break. A telomere-binding protein that is important to maintain this loop structure is telomeric repeat binding factor 2 (TRF2). Inhibition of the TRF2 function causes the telomere structure of the cell to be destroyed, which is the same as the condition of shortened telomeres, inducing cellular senescence or cell death. Introduction of a suppressor type TRF2 into human cultured vascular cells caused their cellular senescence without delay, leading to endothelial dysfunction such as expression of eNOS, reduced activity, and accelerated expression of cytokines and intercellular adhesion molecule-1 (ICAM-1)¹⁴⁾.

Insulin Signal and Vascular Senescence

Calorie-intake restriction improves longevity of various species, from yeast to mice. Low calorie intake prevents a variety of age-related changes, including among others cancer progress, arteriosclerosis, reduced immune capacity, and inflammatory changes. Since calorie restriction decreases plasma glucose and insulin levels, changes in the signal pathway related to these parameters, in conjunction with low calorie intake, may improve longevity. In fact, reduction of signals in this pathway has been reported to elongate the life span of yeast, nematodes, drosophila, and mice¹⁵⁾. Akt phosphorylates the forkhead transcription factor and thereby controls its activity, and it has been found that increased activity of this transcription factor is essential to prolonging longevity due to reduced insulin/Akt signals.

In human beings, this signal pathway has been found to play an important role in controlling the lifespan of human vascular endothelial cells¹⁶⁾. The activity of Akt increased as vascular endothelial cells aged. When this increase was inhibited by introduction of a suppressor type Akt, the lifespan of these cells was elongated. In contrast, introduction of an active type Akt shortened the lifespan of vascular endothelial cells and induced p53 or p21. It has also become evident that when the activity of the forkhead transcription factor is reduced, reactive oxygen species (ROS) within cells increase, which then activates p53. Both increased ROSs and p53 activation are important in cellular senescence caused by insulin/Akt. These findings also indicate that the senescence-controlling signal pathway of nematodes is preserved in human endothelial cells. As activation of Akt is noted

also in arteries of type 2 diabetic mice with hyperinsulinemia and human coronary sclerosis, acceleration of cellular senescence due to insulin/Akt signals may be involved in arteriosclerosis accompanying diabetes or other relevant disease.

Aging and Endothelial Functions: Roles of NO, Vascular Endothelial Progenitor Cells, and Oxidative Stress

Vascular Endothelial Dysfunction

Nitric oxide (NO) is a key player in arteriosclerosis, as indicated by the fact that in 1998, a Nobel prize was awarded for the discovery of research on the role of NO and research on NO metabolism. In the nearly 30 years which have passed since the start of research on vascular endothelium, a massive amount of knowledge and information on this subject has accumulated, and even now, new discoveries and possibilities regarding both the basic and clinical aspects of vascular endothelium are being reported. For arteriosclerosis, it has been clarified that vascular endothelial functions control the vascular functions themselves and play important roles in the development, persistence, progress, and collapse of the disease. In particular, vascular endothelial dysfunction attracts attention as the first stage of arteriosclerosis. Accordingly, control of vascular endothelial functions is extremely important in clarifying the clinical significance of vascular endothelial dysfunction as the first stage of arteriosclerosis. Control of vascular endothelial cell function is also important in understanding the mechanism of endothelial cell dysfunction, and as a prognosis-determining factor and a treatment target for arteriosclerosis. Although diseases such as hypertension, hyperlipidemia, and diabetes, as well as factors such as lack of physical exercise, smoking, excessive intake of salt, and menopause contribute to vascular endothelial impairment, the greatest factor for vascular endothelial dysfunction is aging. Aging is also the most powerful factor for acceleration of arteriosclerosis. In the context of vascular senescence, so-called calendar age should be distinguished from vascular senescence related to arteriosclerosis. In any case, it has become clear that vascular endothelial dysfunction mediated by oxidative stress plays an important role in vascular senescence. Understanding the vascular status (*i.e.* evaluating vascular age) is extremely important in identifying the disease condition, etiology, and progression of arteriosclerosis and also in formulating therapeutic strategies. What is required is not a mere improvement of vascular impairment related to calendar age, but a wider sense of Anti-Aging.

An important mechanism common to endothelial dysfunction in lifestyle-related diseases is involvement of oxidative stress. Oxidative stress is involved in arteriosclerosis by directly damaging vascular endothelial cells and thereby reducing the biological activity of NO, and by activating the intracellular information transmission system (redox reaction) in the vascular wall cells. Reactive oxygen species activate redox transcription factors and accelerate expression of redox genes, by which they induce the proliferation, hypertrophy, wandering and inflammation of the vascular wall cells, apoptosis, and vascular remodeling. NADPH oxidase is the most important source of reactive oxygen species in the blood vessels and is activated under stimulation by various cytokines, vasoactive substances, shear stress, and other relevant stimuli¹⁷⁾. The flow from lifestyle-related diseases,

accelerated oxidative stress, and vascular endothelial dysfunction through to the onset of arteriosclerosis results in a vicious cycle, leading to persistence, progression, and collapse as arteriosclerosis. It is also known that in all lifestyle-related diseases, NO production itself decreases.

Vascular Endothelial Dysfunction and EPC

The reversibility of vascular endothelial dysfunction is an important issue. Vascular endothelial dysfunction can be improved with adequate pharmacotherapy, replacement therapy, change of lifestyle-related habits, or other relevant intervention. Angiotensin receptor antagonists, angiotensin-converting enzyme inhibitors, statins, and thiazolidine derivatives have been reported to have pleiotropic effects, that is, they exert vascular protection effects and achieve direct improvement of endothelial functions in addition to their original effects¹⁸⁾. Replacement therapy with vitamin C, which displays an antioxidative effect¹⁹⁾; L-arginine, which is a substrate of NO and an essential amino acid²⁰⁾; and tetrahydrobiopterin (BH4), which is a coenzyme of NO synthase²¹⁾ improves vascular endothelial functions, as does estrogen replacement therapy in women²²⁾. Changes in lifestyle-related habits, such as performing an adequate amount of aerobic exercise, reducing body weight, stopping smoking, and restricting salt intake contribute to the recovery of endothelial functions.

Vascular endothelial progenitor cells (EPCs) were discovered by Dr. Takayuki Asahara and his associates (Department of Regenerative Medicine, Institute of Biomedical Research and Innovation Laboratory, Kobe-city, Hyogo). It has been reported that when endothelial functions are damaged, EPCs are recruited and involved in regeneration of blood vessels. Researchers administered EPCs to patients with arteriosclerosis obliterans (ASO) of the legs as neovascularization therapy, and remarkable improvement of endothelial functions was observed in addition to neovascularization²³⁾. These results are interesting, as they suggest that endothelial functions may be improved even in patients with serious peripheral ischemic diseases.

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