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REVIEW ARTICLE

Glycative Stress and the progression of Alzheimer-type Dementia: From the Perspective of Amyloid-beta Clearance

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Abstract

Glycative stress is a conception of a state that causes an excess of carbohydrate-derived or fatty acid-derived short-chain aldehydes in the body. Short-chain aldehydes, which are highly reactive, undergo a carbonylation process with lysine and arginine residues in proteins and form advanced glycation endproducts (AGEs). When postprandial hyperglycemia occurs, open-ring glucose with exposed aldehyde groups (-CHO) increases and various carbohydrate-derived aldehydes are formed simultaneously (this phenomenon is named "aldehyde sparks"). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is an enzyme abundant intracellularly as the keystone of defense against glycative stress and plays a role in metabolizing highly toxic glyceraldehyde, however, under diabetic conditions, the enzyme activity is markedly reduced by forming S-(2-succinyl) cysteine-GAPDH. We also found that a high-fat diet reduced GAPDH activity and increased glyceraldehyde and methylglyoxal. High animal fat diets in particular require caution because they increase preference dependence on animal fat. In the lipid-rich brain, they promotes the formation of lipid-derived AGEs formed by methylglyoxal and acrolein providing evidence of their close association with amyloid cascade, ultimately leading to the onset of Alzheimer-type dementia. Through the process of amylod beta (Aβ) glycation modification and cross-linking, Aβ polymerization is promoted and deposited in the brain. Thus, neurotoxicity is aggravated. Furthermore, Aβ progresses toward being persistent and Aβ clearance is reduced. Tau proteins similarly undergo glycation modification and trigger polymerization. methylglyoxal or acrolein-derived glycated Aβ clearance by primary microglia cultured cells reduced significantly compared to the unglycated Aβ provides evidence of the reduced clearance upon glycation. It was also shown that melatonin, which promotes glycolytic cross-linking degradation, may promote microglial Aβ phagocytosis. We next plan to examine the potential of plant extracts that promote glycation cross-linking degradation to improve Aβ phagocytosis. Advanced Alzheimer-type dementia with a disoriented neural network hardly recovers with treatment. In this review article, we discussed possible future therapeutic strategies for Alzheimer-type dementia by increasing Aβ clearance from the early stage such as the prevention of Aβ-glycation and the promotion of glycated-Aβ degradation, which may be a paradigm shift.

Introduction

Glycative stress refers to a series of phenomena where excessive aldehydes cause the formation of carbonyl compounds and advanced glycation endproducts (AGEs) and the reduction in functionality of proteins, and lipids; aldehydes are derived from excessive ingestion of reducing sugar, *i.e.*, glucose, and fructose, and alcohol, which induced the unusual modification of the protein, lipid, DNA base, and amyloids.[1] The formation and accumulation of AGEs in different body parts increase with age and most of the time this process remains irreversible. The AGEs either change the function of the macromolecules or make them totally non-functional, in some instances, the body's immune system recognizes them as foreign materials due to structural changes. Processing of the macromolecules by different cell types also alters due to the cross-links of AGEs. Some conditions like diabetes, dyslipidemia, and alcohol dependence could multiply the level of glycative stress. Furthermore, glycative stress causes diverse diseases such as osteoporosis, Alzheimer-type dementia (AD), skin aging, ovarian function decline, age-related macular degeneration, hair aging, and sarcopenia/dynapenia, and induces age-related regressive changes.[1-3]

Diabetes is a typical disease where glycative stress is intensive and its incidence rate is rising in Japan as well as worldwide presently. Today is "exactly when we fight against glycative stress due to dramatic changes in our food habits and life style." AD pathogenesis is usually initiated by the cleavage of Aβ from it's precursor protein, then it's deposition and accumulation; followed by amyloid plaque formation. Glycative stress can enhance the formation of crosslinks between proteins and thereby can trigger amyloid plaque formation. The present study reviews the effects of glycative stress on Aβ clearance by cellular systems focusing on AD.

What is glycative stress?

The concept of glycative stress is demonstrated in **Figure 1**. Causative factors are **i**) glucose spikes, **ii**) high animal-fat diet (high triglyceride level, high LDL cholesterol level), and **iii**) a high rate of alcohol consumption. These three factors are common in aldehyde. A considerable number of persons are at level of 140 mg/dL or higher for high levels of postprandial blood glucose even when their fasting glucose level is normal. This is designated as glucose spikes. It has been elucidated that glucose spikes induce rapid progression of arteriosclerosis and have risks to cause diverse physical impairments such as cerebro/cardiovascular events.

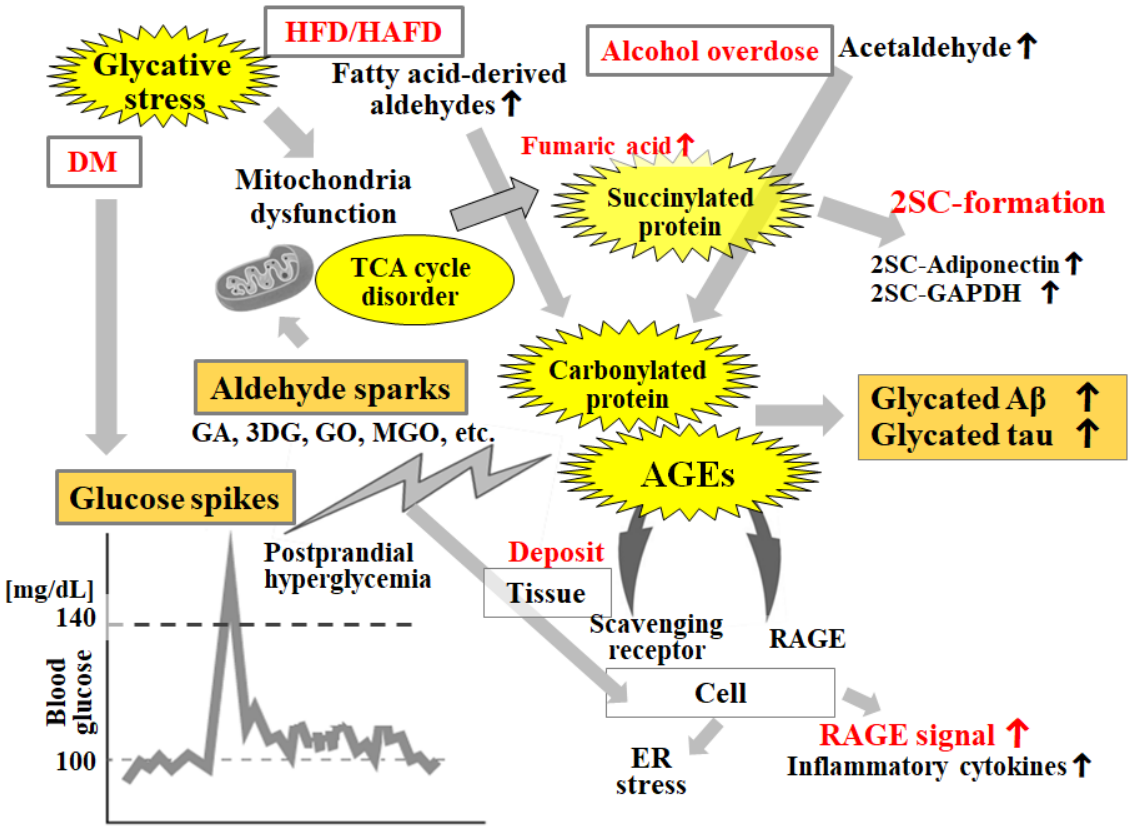

Figure 1. The Concept of glycative stress.

Glycative stress is a situation that causes an excess of carbohydrate-derived or fatty acid-derived short-chain aldehydes in the body. It induces glycated Aβ production in the brain in Alzheimer's disease. Aβ, amyloid beta; AGEs, advanced glycation endproducts; RAGE, receptor for AGEs; DM, diabetes mellitus; HFD, high-fat diet; HAFD, high animal fat diet; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; 2SC, S-(2-succinyl) cysteine; GA, glyceraldehyde; 3DG, 3-deoxyglucosone; GO: glyoxal, MGO: methylglyoxal; ER, endoplasmic reticulum.

Glucose spikes trigger "aldehyde sparks." This is a phenomenon that causes a chain reaction for the simultaneous generations of diverse types of aldehydes at the moment when a new aldehyde was formed. This concept was created by the inspiration of results of our preliminary experiment to measure aldehydes. Spike and Recovery Test confirms a degree of accuracy; solution prior to addition (content X) + addition (content Y) → solution posterior addition (content Z): when the value of Z/X+Y is close to one, reliability is high. However, the results of the recovery rate in our experiment were unexpectedly high and there were cases where the recovery rates of aldehydes were several times or several score times high in Table 1.[4] Oral glucose tolerance test demonstrated elevations in 3-deoxyglucosone (3-DG), glyoxal (GO) and methylglyoxal (MGO).[5] This findings matched the phenomenon of "aldehyde sparks."

Table 1. Aldehyde measurement by DAN-labeled HPLC; Spike and Recovery Test.

	AA		3DG		GA		GO		MGO	
【HSA+Glucose solution】										
Addition (+) (pg)	1,008.3	± 21.0	83,538.2	± 625.6	536,946.9	± 20,489.0	1,317.7	± 28.7	1,794.4	± 24.7
Addition (–) (pg)	607.0	± 22.4	59,095.6	± 816.8	89,058.6	± 2,997.0	699.9	± 10.6	211.1	± 3.1
Difference (pg)	401.3		24,442.6		447,888.3		617.8		1,583.3	
Additional dosage (pg)	400.0		25,000.0		100,000.0		600.0		250.0	
Recovery rate (%)	100.3		97.8		447.9		103.0		633.3	
【HSA solution】										
Addition (+) (pg)	475.7	± 40.9	2,048.7	± 22.6	3,545.2	± 262.0	17.9	± 0.8	41.8	± 0.9
Addition (–) (pg)	468.5	± 28.5	1,311.9	± 36.4	303.2	± 36.3	11.1	± 1.9	11.1	± 0.6
Difference (pg)	7.2		736.8		3,241.9		6.8		30.7	
Additional dosage (pg)	10.0		700.0		2,000.0		10.0		5.0	
Recovery rate (%)	72.2		105.3		162.1		67.9		614.3	
【Plasma】										
Addition (+) (pg)	516.5	± 23.1	3,393.0	± 705.2	720.4	± 207.6	143.5	± 11.5	33.6	± 2.2
Addition (–) (pg)	342.2	± 24.5	2,055.7	± 87.2	167.6	± 7.9	97.2	± 1.3	12.8	± 0.7
Difference (pg)	174.3		1,337.3		552.8		46.4		20.9	
Additional dosage (pg)	200.0		500.0		500.0		50.0		7.0	
Recovery rate (%)	87.2		267.5		110.6		92.8		297.9	

Results are expressed as mean ± standard deviation (n = 3). The additional solution consists of the mixture of AA, 3DG, GA, GO, and MGO. Each dosage is presented in this table. DAN, 2,3- diaminonaphthalene, HSA: human serum albumin, AA: acetaldehyde, 3DG: 3-deoxyglucosone, GA: glyceraldehyde, GO: glyoxal, MGO: methylglyoxal. The table quoted from Reference 4.

Reactions of the Aldehyde group (-CHO), which is highly reactive, progress even without any enzymes. Progression of reactions are prescribed by substrate concentration, temperature, and time.

Glucose has a cyclic form in a natural state. When a portion of glucose opens the ring, it exhibits an open-chain form, exposing the aldehyde group (-CHO), which exerts a toxic aldehyde effect.[6] The problematic factor in glucose spikes is the open-chain structure. The ratio of the opening ring in glucose is as low as 0.002%. Within normal blood glucose levels (90–140 mg/dL), deleterious effects, which are designated as glucotoxicity, are suppressed to a limited extent, preventing harm from reactive aldehyde exposure. The level of GA (2–20 μM)[5] is higher than that of open-chain glucose and the contribution to the formation of pathogenesis is significant.[6]

Typical carbohydrate-derived aldehydes, which induce glycation, are glyceraldehyde (GA), glycolaldehyde, acetaldehyde (AA), 3-DG, GO, and MGO. In the lipid-rich brain, fatty acid-derived aldehydes, *i.e.*, acrolein (Acro), malondialdehyde, and 4-hydroxynonenal (4-HNE), contribute significantly to produce the glycative stress.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) exists accounting for as much as 10–20% of total protein volume in the cytoplasm. GAPDH is regarded as an enzyme that is the key to the preventive system against glycative stress to metabolize GA for living creatures.[6–9] GA that enters into cells are phosphorylated and converted into glyceraldehyde-3-phosphate (GAP), which stays inside the cell without leakage to the outside. In the glycolysis system, GAP is converted by GAPDH to 1,3-bisphosphoglycerate, which is further metabolized to pyruvate and finally to lactic acid, followed by excretion from the cell. This reaction progresses through the oxidizing agent, NAD^+. When the ratio of NAD^+/NADH decreases, reactions do not proceed. This reaction generates NADH and later it conveyed to mitochondria to reduce oxygen and being oxidized by NAD^+. This performs the function of maintenance of the ATP cycle.

High blood glucose and high fat diet (HFD) induce the decline or depletion of NAD^+, which could damage the TCA cycle. Subsequently, fumaric acid increases and succinylation of GAPDH is caused (the formation of 2SC-GAPDH).[10–13] 2SC-GAPDH does not have enzyme acitivity and cannot metabolize GAP. Thus, GA concentrations elevate inside and outside cells. [12] It is recognized that 2SC-adiponectin (monomer and trimer) increases in adipose tissues, inhibiting the formation of the highly membrane-permeable hexamer and decreasing adiponectin blood levels.[11] Consequently, insulin resistance is exacerbated and blood glucose spikes ocurr more freaquently.

Once intracellular GAPDH activities are inhibited by koningin acid (KA), GA concentrations elevate in cells and culture medium. Eventually, cells are damaged to fatal levels (**Figure 2**).[6, 14] In experiments of mice fed with HFD, GAPDH activities in the liver deteriorated and GA in blood increased.[14] GAPDH activities as a glycolytic

enzyme decrease and GAP or its isomer, dihydroxyacetone phosphate, which are substrates of GAPDH, are not metabolized and are deposited. From these metabolites, short-chain aldehydes, *i.e.*, GA, MGO, are formed.[15]

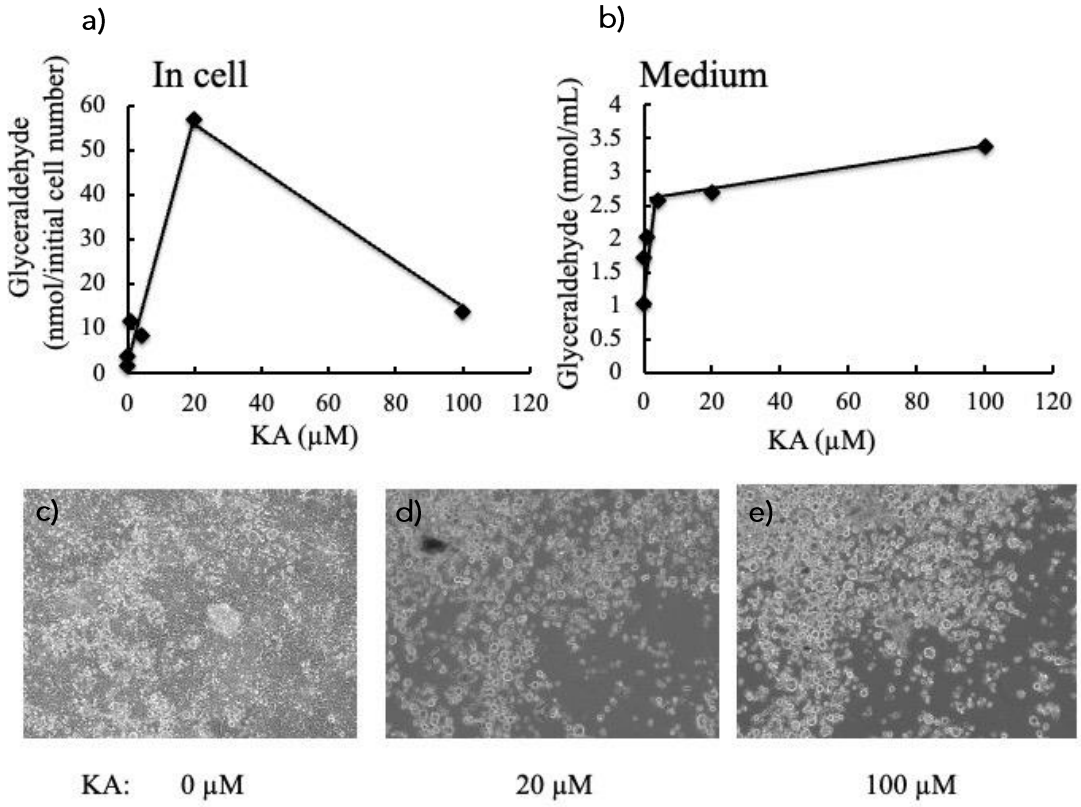

Figure 2. Effect of KA on HCT116 cell intracellular and medium GA.

HCT116 cells (1 x 10^5) were inoculated on 24 well plate and incubated in a high glucose DMEM medium containing antibiotics and 10% fetal bovine serum. KA was added to the medium one day after inoculation. Cells were harvested 7 days after inoculation and washed with the medium. Intracellular GA was extracted with 75% ethanol. The intracellular (**a**) and medium (**b**) GA was quantified by the method of Martin-Morales *et al.* (Reference 14). Cells after 6-day incubation with KA (**c**: 0 μM, **d**: 20 μM, and **e**: 100 μM) were photographed (lower). Figures quoted from Reference 6). KA, koningin acid; GA, glyceraldehyde.

Carbonylated protein and AGEs are taken into cells through scavenger receptors. These are causative factors of endoplasmic reticulum (ER) stress.[1] Furthermore, aldehydes directly move into cells and modify intracellular proteins. The chemical bond between AGEs and receptors for AGEs (RAGE) generates, via intracellular signal activation, inflammatory cytokine (tumor necrosis factor α: TNF, interleukins).

Effects of high animal-fat diet on the brain

Among high-fat diets (HFD), excessive ingestion of animal fat with a high animal-fat diet (HAFD) is an interest from the view of dependence. HAFD induces the elevation of leptin resistance, attenuating the function of the adipocyte-derived hormone, leptin. It is widely known that difficulty in losing weight is the result.[16, 17] The arcuate-nucleus located in the appetite center of the hypothalamus, is considered a major site for the regulation of homeostasis of appetite, which hormones and the autonomous nervous system are responsible for (a metabolic/hunger control system). HAFD, however, causes, via ER stress and oxidative stress, the inflammation of the hypothalamus could paralyze the metabolic/hunger control system. Therefore, correct judgments of the brain are not delivered regarding the amount of caloric requirement for individuals, and concurrently individuals have a habit of avoiding exercise.

In the HAFD-fed mice, the infiltration of activated microglia with inflammation was rapidly observed in the hypothalamus, and damage to the brain as well as leukocyte migrations occurred. Due to this, the brain was in a state of chronic inflammation.[18] However, by doing regular exercise, the mouse had drastic improvements in the inflammation of the brain, which had been induced by HAFD.[19]

There are attentive interest in the similarity of dependence between animal fat, and drugs, nicotine, and alcohol. In diverse types of dependence, it is common that the ingestion volume of stimuli increases, as the reward sensitivity decreases; the stimulus recognition threshold in the brain reward system gradually elevates and the brain is unable to gain satisfaction and pleasure (reward) with the same amount as before.

Rats fed with a high-calorie diet in a mixture of animal fat and sucrose behave like drug-addicted rats with the ingestion of cocaine or heroin, a fat amount satisfactory for the brain reward system increases, and the brain reward of the ingestion of food is difficult to attain. Thus, they were in a vicious circle where "the brain cannot be satisfied no matter how much they eat."[20]

A hypothesis is examined that fat acid-derived aldehyde and its protein modification would be related to the mechanism where HAFD causes ER stress on nerve cells with the metabolic reward system. Lipid that is contained in the brain tissues in abundance is a supplier of fatty acid-derived aldehydes. HAFD provides an intensive glycative stress to the brain and concurrently is addictive. Therefore, countermeasures against HAFD are critically important to prevent the onset and progression of AD. γ-Oryzanol contained in unpolished brown rice is expected to be effective regarding efficacy on HAFD dependence.[21,22]

Glycative stress and AD

Neuropathological characteristics of the brain of AD patients consist of cerebral atrophy, senile plaque, and neurofibrillary tangle. Senile plaques consist of Aβ. Aβ is a peptide with a molecular weight of 4,300–4,500, consisting of approximately forty

amino acids (Aβ40, Aβ42). Aβ is generated from amyloid-beta precursor protein (APP) through the excision with β- and γ-secretase (Figure 3).

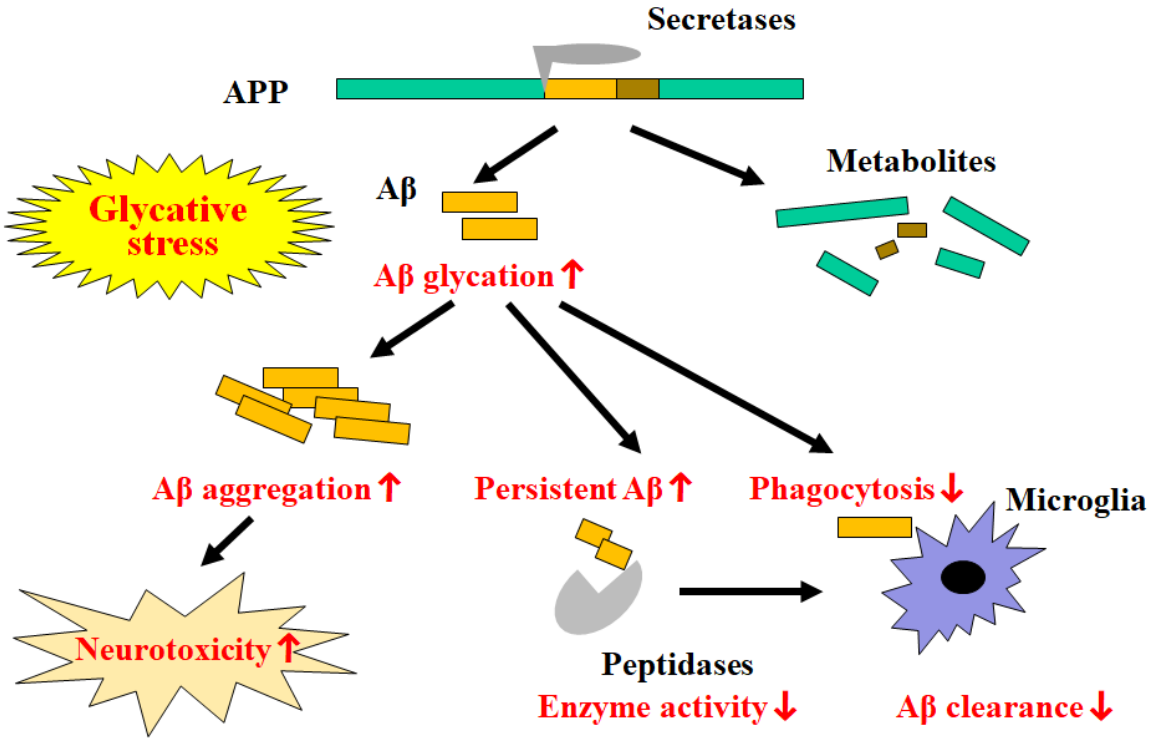

Figure 3. Effects of glycative stress on the amyloid beta (Aβ) cascade.
APP, amyloid precursor protein; Aβ, amyloid beta.

Normally, Aβ is rapidly degraded and discharged as brain wastes (Aβ clearance). However, when abnormal Aβ is generated due to modification of Aβ structure by oxidative and glycative stress, insoluble fibers are formed and deposited.[23, 24] Specifically, protein cross-linking is formed, which induces amyloid β-peptide polymerization. This formation of abnormal Aβ polymer causes resistance to degradation, elevated neurotoxicity and decline of Aβ clearance. Furthermore, when Aβ is not discarded and is accumulated in the brain, Aβ senile plaques (amyloid pathology) are generated. Neurotoxic abnormal Aβ clings to healthy nerve cells, which may affect nerve cells negatively. With this as a trigger, tau protein, which is a microtubule-connected protein, induces the formation of neurofibrillary tangle with filamentation and condensation in cytoplasm. As a result, the brain gradually atrophies and AD progresses. This is the concept that unites glycative stress with the "amyloid cascade hypothesis."[25-27]

Glycative stress significantly affects the Aβ cascade. The trigger point is a state where glucose with an open-chain form as well as short-chain aldehyde increases. These aldehydes, as carbonyl stress, induce a carbonylation reaction in proteins. Further, aldehydes generate AGEs and contribute to

the neurodegeneration of AD.[28-31] It is possible that the glycation of Aβ by aldehydes is a detrimental morphological change with toxicity enhancement.[30]

MGO is associated with the onset of AD.[32-36] MGO increases the size of Aβ aggregate.[32] AD patients have higher MGO concentrations in cerebrospinal fluid (CSF) than persons with no physical or mental disability. [33] and similarly have more MGO-derived hydroimidazolone compounds.[34] It is reported that the higher MGO concentration in serum is, the more cognitive function decreases in the progression of dementia.[35]

On the other hand, Acro is associated with the onset of AD.[37-44] Acro, which is generated through the lipid peroxidation and the metabolism of polyamine, is highly nucleophilic and strongly reactive. Acro induces the carbonylation of protein, performing Michael addition with nucleophilic amino acid, *i.e.*, cysteine, lysine, and histidine. It was observed that rats with oral administration of Acro (2.5 mg/kg/day, intragastric administration for eight weeks) had a decline in cognitive functions and hippocampal atrophy.[39] Clinically, Acro increases in the hippocampus and the temporal lobe of AD patients.[37] Further, Acro binding protein in plasma and cerebrospinal fluid is higher than in persons with no physical or mental disability.[38, 41] Acro is related to the onset and progression of AD.[40, 42]

When Aβ is incubated under the presence of glucose, it is glycated and caused AGE modification. Aβ has lysine residues at the number 16 and 18 of amino acid sequence (Lys; K) and arginine residue at the number 5 (Arg; R). These amino acid sequence positions are related to glycation.[31] Subsequently, Aβ is incubated under the existence of glucose and is aggregated. It is possible that Aβ-AGE is a "seed" that promotes the aggregation of Aβ by crosslink formation among Aβ peptides.[32] AGE formation due to Aβ glycation changes Aβ aggregation potential.[44] Aβ glycation remarkedly retards β conversion to mature fibrils, and toxic forms such as oligomer are inclined to remain.[32. 45]

It is reported that regarding protein in frontal lobe tissues containing protein of senile plaque, AD patients have three times as much AGEs as healthy-aged people.[46] Whereas, when the Aβ-glucose reaction system is incubated with the addition of aminoguanidine, an inhibitor against glycation, the formation of Aβ aggregation is reduced.[46] The existence of AGEs, *i.e.*, pentosidine, pyrraline, and N^{ε}-carboxymethyl lysine (CML), is observed in senile plaque.[47, 48] There is a possibility that Aβ-AGE, where Aβ is altered due to glycation, is related to factors for the formation of protein cross-linking and the promotion of Aβ aggregation.

Tau protein of paired helical filaments (PHF), which was collected from brains of AD patients, was examined and tau protein that was modified by AGEs was observed, while soluble tau protein collected from persons without AD or cognitive impairment did not show AGE modification. It is reported that tau protein has thirteen lysine residues of amino acid sequence and six lysine residues are glycated out of thirteen.[48] As glycation positions of tau protein are located in binding sites with microtubules, binding functions are

damaged. Glycated tau protein induces oxidative stress, thus forming active oxygen and IL-6, and nerve cell functions are damaged.[49]

In an *in vitro* experiment system of tau protein, it is demonstrated that glycated tau protein generates PHF-like fibers but non-glycated tau protein does not generate fibers.[50] AGEs such as pentosidine, pyraline and CML are observed in PHF. Glycated tau protein induces the formation of APP and Aβ. Abilities of intracellular transport are reduced due to the phosphorylation and glycation of tau protein, and APP cannot be secreted to the outside, resulting to cause APP deposition in cells. The glycation of Aβ and tau protein facilitatively affects the deposit and aggregation of Aβ and concurrently exacerbates the deposit of tau.

In microglia and astrocyte, which exist in brains of AD patients, pentosidine, CML and RAGE exist and they increase in comparison with healthy aged people.[51, 52] Microglia is a type of cell, which is designated as resident macrophage in the central nerve system. Macroglia possesses functions of neuron removal and repair. Activation of microglia is linked to the generation of inflammatory cytokines such as tumor necrosis factor (TNFα), interleukin-1β (IL-1β), and interferon (IFN), which is speculated to induce impairments of nerve cells.[53] Astrocyte is a star-shaped glial cell, which specifically exists in the brain and the spinal, performs the maintenance of functions in the central nervous system to control the bloodstream, supply energy to nerve cells, participate in synapse functions and regulate neurotransmitters.[54] When chicken egg albumin-AGE is added to cultured cells, formations of nitric oxide (NO), IL-6, and TNF-α. are observed with RAGE mediation.[53, 55]

An experiment, where diverse AGEs are added to a culture system of rats' cerebral cortical neuron, confirmed that GA-derived AGEs shows more intensive apoptosis induction than Amadori products, glycolaldehyde-derived AGEs, MGO-derived AGEs, and GO-derived AGEs.[56] In contrast, CML does not induce cell death. These findings suggest that the formation and aggregation of AGEs, which is related to Aβ in brains and senile plaque accumulations, induce the neurotoxicity acquisition of Aβ, inflammations with RAGE mediation, and apoptosis. Therefore this may be related to the promotion and enhancement of neurocyte death in the pathogenesis of AD. [57]

It is considered that the glycations of not only Aβ but also tau protein and α-synuclein are related to the onset and progression of dementia in the cranial nervous system.[31]

Glycative stress and Aβ clearance

The formation of Aβ occurs in spite of age and the presence/absence of AD. In normal brains, Aβ is removed in an appropriate manner and is not deposited or slightly deposited.[58] Aβ deposit that is recognized in pathological anatomy reflects the balance among the formation, deposit, and removal of Aβ at that point. Brains of AD patients with pathological anatomy present an accumulation of activated microglia in the periphery of senile plaque, as is confirmed. It

is suggested that impairments of Aβ clearance is related to this.

It is considered that γ secretase is related to cleaving ends of carboxy in the Aβ formation pathway and determines the volume and cohesive property of Aβ. Therefore, γ secretase is considered to be an important potential drug target for AD. Multiple low molecular weight compounds have been examined as γ-secretase inhibitors. However, approaches in methods of treatments to inhibit the formation of Aβ have experienced failures in the process of clinical trials. This suggests that Aβ may have a physiological significance and the complete removal of Aβ in the brain could not lead to an AD cure.[59]

We understand that the maintenance of Aβ homeostasis is essential for the control of AD progression and have started examining the impacts of glycative stress on the phagocytic activities of microglia. There have been no reports regarding impacts on the phagocytosis of microglia when Aβ receives post-translational modifications. Therefore, the present study analyzed alternations in phagocytic activities of microglia toward glycated Aβ. Glycated Aβ was prepared through treatments of MGO and Acro. The result showed that microglia phagocytosed Aβ, while functions of phagocytosis toward glycated Aβ remarkedly declined (**Figure 4**).[60] The present findings suggest that "microglia phagocytosis resistance toward glycated Aβ" is one of the mechanisms of the degradation of Aβ clearance due to glycative stress. Our experiment that employed rat-derived primary microglia cells observed that the Aβ addition group showed superior growth and proliferation of cells compared with that of the non-Aβ addition group. These findings, which should be called a "gliatrophic action", maybe one of the physiological significance of Aβ. Further studies are expected to produce research results for this case.

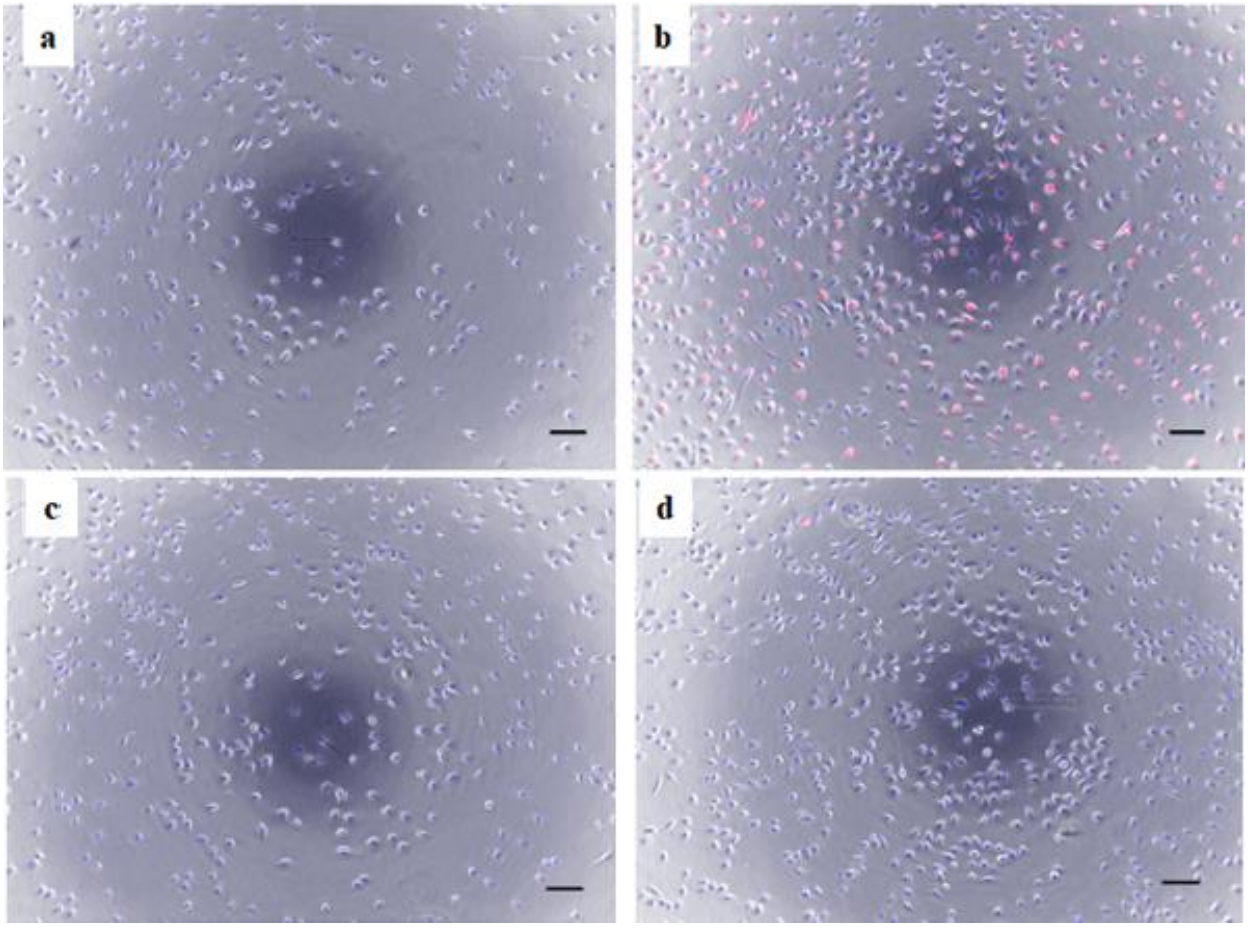

Figure 4. Aβ phagocytic activity of microglia: Fluorescence microscopy images.
a) Aβ non-addition, b) Aβ addition, c) MGO-treated glycated-Aβ addition, d) Acro-treated glycated-Aβ addition. Red-stained cells indicate Aβ-phagocyted microglia. Red-fluorescence-labeled Aβ (TAMRA-Aβ) and rat-derived primary microglia (Cosmo Bio, Otaru, Hokkaido, Japan) were used. The bar indicates 200 μm. Aβ, amyloid beta; MGO, methylglyoxal; Acro, acrolein. The figure quoted from Reference 60.

Countermeasures against glycative stress as AD prevention and treatment strategies

Several studies reported on improvement factors of Aβ clearance for methods to prevent the development of AD.

Galantamine, which is an inhibitor against acetylcholine esterase (AChE), binds with nicotinic AChR on the surface of microglia as a ligand, and facilitates the expression of CD14/TLR complex and promotes Aβ clearance.[61] Sodium rutin, through the expression of phagocytosis-related receptor for microglia and the increase of cell population, promotes Aβ clearance.[62] Further, rutin (quercetin-3-o-rutinoside) has anti-oxidative and anti-glycative activities (inhibitory effcts on FGES formation) and it could perform suppressive functions of polymerization due to Aβ glycation in the Aβ cascade.

Glycation cross-linking is related to the polymerization of Aβ. Therefore, expectations are rising for functional food that has cleaving effects on glycation cross-linking (effects to accelerate AGE degradation).[63-67] Extracts of water chestnuts (*Trapa japonica,* hishi in Japanese),[64] rosemary (*Salvia rosmarinus*),[65] *Kuromoji* (*Lindera umbellata*)[66] and black galangal (*Kaempferia parviflora*)[67] have functional ingredients with promotive effects on AGE degradation. Specifically, extracted materials of rosemary (main component: rosmarinic acid) is reported to have alleviating activities on dementia.[68] The promotive effects on AGE degradation would be related to its mechanism. Plant extracts and compounds[64, 66, 67, 69] with glycation cross-linking cleaving effects[70] are shown in **Table 2**. We next plan to test their potential to improve Aβ phagocytosis.

Substances	Form	Concentration	Cleavage activity (%)
Natural products	Water chestnut (*Trapa bispinosa* Roxb.)[64]	1 mg/mL	32.5
	Kuromoji (*Lindera umbellata* Thunb.)[66]	1 mg/mL	8.1
	Black galangal (*Kaempferia parviftora* Wall.ex.Baker)[67]	3.9 mg/mL	6.8
Purified substance[69]	Melatonin	0.4 mmol/L	15.8
	Urolithin B		14.9
	Rosmarinic acid		9.0
	Carnosic acid		1.9
	Quercetagetin		1.3
	Scutellarein		1.2
	Epigallocatechin gallate		8.8
	Epigallocatechin		8.5
	Gallocatechin		8.8
	Catechin		5.1
	Epicatechin		3.6
	Epicatechin gallate		2.0

Table 2. Cleavage activity of AGE crosslinking.

Cleavage activity; The cleavage activity of AGE crosslinking rate was calculated according to the method of Vasan S *et al.* (Reference 70), based on the fact that 1 mol of 1-phenyl-1,2-propanedione (PPD) in the reaction solution yields 1 mol of benzoic acid when the α-diketone bond of PPD.

The key measures against glycative stress are dietetic treatment and exercise therapy. Stratified dietary is classified into three phases as follows: ⅰ) control of blood glucose spikes and aldehyde trapping, ⅱ) inhibition of AGE formation and ⅲ) promotion of AGE degradation. We recommend efficient ingestion of food ingredients that have these effects. For carbohydrate, which accounts for approximately 60% of total caloric intake, we recommend whole-grain foods. Especially to counter animal fat dependence, γ - oryzanol, which contains in unpolished brown rice, could exert effects.[16, 17, 21, 22]

The deterioration of "sleep quality" and the disruption of circadian rhythm raise risks of AD onset. [71] Melatonin is secreted during night-time sleep and has anti-oxidative and anti-glycative activities (promotive effects on AGE degradation), [69] playing a role protect brains during sleep from oxidation and glycation. It is reported that melatonin has effects of the inhibition of Aβ formation,[72] the inhibition of Aβ aggregation,[73-76] the alleviation of neurotoxicity,[77, 78] enhancement of Aβ lymphatic clearance,[79] and the maintenance of memory function.[80, 81] Therefore, lifestyle guidance to improve "sleep quality" and increase melatonin secretion should be included to prevent the progression of AD.

Conclusion

Aβ plays a critical role in the onset and progression of AD. The physiological roles of Aβ, however, have not been explored. As previously mentioned, Aβ aggregation induces the elevation of neurotoxicity and the deposit to the brain. Consequently, Aβ clearance deteriorates. It is difficult to cure completed AD and recovery of advanced mental dysfunction cannot be expected. Further, in the present stage, the physiological roles of Aβ are unknown. From this point of view, grave risks are involved in therapies aiming to complete Aβ elimination. Therefore, as primary protective methods against glycation, improvements in lifestyle habits regarding diet, exercise, and "sleep quality" must be a focus for countermeasures against AD. A paradigm shift must be taken to protect homeostasis of Aβ cascade and Aβ clearance, implementing measures against glycative stress from an early stage.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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