Research Article

Microglial Amyloid-β1-40 Phagocytosis Dysfunction Is Caused by High-Mobility Group Box Protein-1: Implications for the Pathological Progression of Alzheimer’s Disease

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In Alzheimer disease (AD) patient brains, the accumulation of amyloid-β (Aβ) peptides is associated with activated microglia. Aβ is derived from the amyloid precursor protein; two major forms of Aβ, that is, Aβ1-40 (Aβ40) and Aβ1-42 (Aβ42), exist. We previously reported that rat microglia phagocytose Aβ42, and high mobility group box protein 1 (HMGB1), a chromosomal protein, inhibits phagocytosis. In the present study, we investigated the effects of exogenous HMGB1 on rat microglial Aβ40 phagocytosis. In the presence of exogenous HMGB1, Aβ40 markedly increased in microglial cytoplasm, and the reduction of extracellular Aβ40 was inhibited. During this period, HMGB1 was colocalized with Aβ40 in the cytoplasm. Furthermore, exogenous HMGB1 inhibited the degradation of Aβ40 induced by the rat microglial cytosolic fraction. Thus, extracellular HMGB1 may internalize with Aβ40 in the microglial cytoplasm and inhibit Aβ40 degradation by microglia. This may subsequently delay Aβ40 clearance. We further confirmed that in AD brains, the parts of senile plaques surrounded by activated microglia are composed of Aβ40, and extracellular HMGB1 is deposited on these plaques. Taken together, microglial Aβ phagocytosis dysfunction may be caused by HMGB1 that accumulates extracellularly on Aβ plaques, and it may be critically involved in the pathological progression of AD.

1. Introduction

Alzheimer’s disease (AD) is characterized by the deposition of amyloid-β (Aβ) plaques, accumulation of neurofibrillary tangles (NFTs), and loss of synapses and neurons in particular brain areas [1]. Experimental studies using transgenic AD mouse models have demonstrated that Aβ accelerates NFT formation [2, 3] and is closely associated with synaptic damage [4]. In contrast, Aβ reduction in the brain by Aβ immunization restores cognitive functions in transgenic AD mouse models [5–9] and also appears to slow cognitive decline in human AD patients [10]. Thus, the accumulation of Aβ may play a key role in the pathogenesis of AD [11].

Aβ is derived from the sequential proteolysis of amyloid precursor protein (APP) by β- and γ-secretases and is composed of 37–43 amino acid residues because γ-secretase, which is a protein complex including presenilin (PS), generates the C-terminal of Aβ with different lengths [12]. Among the variations in Aβ, Aβ1-40 (Aβ40) and Aβ1-42 (Aβ42) are the major species found in AD brains. The most predominant species deposited in Aβ plaques is Aβ42 [13], which is prone to aggregation [14] and indicates increased neurotoxicity [15]. On the other hand, Aβ40 is the major
soluble species; its secretion is 10-fold more than that of Aβ42 in normal brains. A previous study demonstrated that the deposition of Aβ40 in AD brains is particularly correlated with synaptic and neuronal loss [16]. Thus, lowering the concentration of Aβ40 and Aβ42 in the brain may serve as a disease-modifying therapy for AD patients.

Activated microglia accumulate on Aβ plaques in AD brains. Although microglial accumulation was initially believed to be involved in the formation of Aβ plaques [17], experimental studies later demonstrated the ability of microglia to phagocytose Aβ peptides [18, 19]. In addition, we demonstrated microglial contribution in Aβ42 clearance using primary cultured rat microglia [20–25]. However, it has been reported that microglial dystrophy occurs in aging human brains [26], and the age-related disability of microglial Aβ phagocytosis has been demonstrated experimentally [27]. Thus, the dysfunction of microglial Aβ phagocytosis appears to be closely involved in the progression of AD pathology.

High-mobility group box protein 1 (HMGB1) is an abundant nonhistone chromosomal protein that is released from cells undergoing necrosis [28, 29]. The released HMGB1 behaves like an inflammatory mediator by acting on receptor for advanced glycation end products (RAGE) and Toll-like receptors (TLRs) 2 and 4 [30, 31]. We have previously reported that HMGB1 is extracellularly associated with Aβ plaques in AD brain and is involved in the pathogenesis of AD as an inhibitory factor against microglial Aβ42 phagocytosis by interfering with uptake [32, 33]. However, the effect of extracellular HMGB1 on the microglial phagocytosis of Aβ40, but not Aβ42, has not been elucidated. Therefore, in the present study, we analyzed rat microglial Aβ40 phagocytosis in the presence and absence of exogenous HMGB1.

2. Materials and Methods

2.1. Primary Culture of Rat Microglia and Drug Treatment. The primary culture experimental procedure was reviewed and approved by the Committee for Animal Research at Kyoto Pharmaceutical University. Primary cultured microglia (over 97% pure) were prepared, as described previously [32]. Briefly, brain tissues were isolated from newborn Wistar rats, minced, and gently dissociated by trituration in Dulbecco’s modified Eagle medium (DMEM). The tissue suspension was filtered through a 50 μm mesh into 50 mL tubes, and cells were collected by centrifugation at 200 × g for 10 min. Cells were then resuspended in DMEM with 10% fetal calf serum, 100 units/mL penicillin, and 100 μg/mL streptomycin; they were then plated onto 100 mm diameter dishes at 37°C in humidified 5% CO2/95% air. We then harvested the floating microglia from mixed glial cultures. Microglia were transferred to 24-well plates (3.0 × 105 cells/well) and were allowed to adhere at 37°C overnight; they were then treated with sterilized phosphate-buffered saline (PBS) as the vehicle or synthetic human Aβs (Aβ40 or Aβ42; Anaspec, San Jose, CA) in the presence or absence of calf thymus-purified HMGB1 (WAKO Chemicals, Osaka, Japan). We previously demonstrated that 1 μM Aβ42 for 12 h markedly phagocytosed by rat microglia [20], and 0.3 μM HMGB1 inhibits the phagocytosis [32, 33]. When Aβ40 at 1–3 μM were added into the culture, we could detect Aβ40 phagocytosed by rat microglia by Western blot analysis [25]. Therefore, in the present study, we adopted the concentrations at 1 μM and 0.3 μM for the treatments with Aβs and HMGB1, respectively. To make the experimental conditions more accurate, we dissolved the lyophilized human Aβ peptides in distilled and sterilized water at a high concentration, and small aliquots were kept at −80°C until use. Subsequently, Aβ stock solutions were diluted using sterilized PBS, and once Aβ was thawed, no Aβ was refrozen to eliminate variance due to repeated freezing and thawing.

2.2. Immunocytochemistry. Twelve hours after Aβ treatment, microglia were gently rinsed three times with PBS and then fixed with 4% paraformaldehyde in 100 mM phosphate buffer (PB) for 30 min. Cells were then incubated with a mouse monoclonal antibody against Aβ (clone 6E10, 1 : 1000; Chemicon, Temecula, CA) and a rabbit polyclonal antibody against HMGB1 (1 : 1000; BD Pharimingen, San Diego, CA). The primary antibodies were followed by application of a rhodamine-labeled anti-mouse immunoglobulin (Ig)G antibody and fluorescein isothiocyanate-labeled anti-rabbit IgG antibody (each diluted 1 : 500; Molecular Probes, Eugene, OR). Furthermore, cells were incubated with Hoechst 33258 (1 : 5000; Molecular Probes) to visualize microglial nuclei. Labeled fluorescence was detected using a laser scanning confocal microscope LSM 510 (Carl Zeiss, Jena, Germany).

2.3. Aβ Phagocytosis and Clearance Assay by Western Blot Analysis. Twelve hours after Aβ treatment, microglia and culture media were collected and lysed with Laemmli’s sample buffer and then analyzed by Western blot analysis. Briefly, samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; 20% polyacrylamide gels in Tricine buffer). Proteins were transferred to a polyvinylidene difluoride membrane (Millipore, Billerca, MA) by electroelution and then incubated with a mouse monoclonal antibody against Aβ (clone 6E10, 1 : 2000; Chemicon), followed by a horseradish peroxidase-linked secondary antibody against mouse IgG (1 : 1000; Amersham, Buckinghamshire, UK). Subsequently, protein bands were detected on radiographic films (Kodak, Rochester, NY) using a chemiluminescence kit (ECL kit; Amersham). For semi-quantitative analysis, radiographic films were scanned with a CCD color scanner (Duoscan, AGFA, Mortsel, Belgium) and then analyzed densitometrically using the public domain US National Institutes of Health image 1.56 program.

2.4. Aβ Degradation Assay. Microglia were harvested and resuspended in 100 mM Tris-HCl buffer (pH 7.5) containing 10 mM KCl, 1.5 mM MgCl2, and 1 mM DTT and then homogenized. After centrifugation (50,000 × g) for 20 min at 4°C, the protein concentration of the supernatant was measured and used as the microglial cytosolic fraction.
The Aβ peptide (3 μM Aβ40 or Aβ42) was incubated with the microglial cytosolic fraction (final concentration of 1 mg/mL) in the presence or absence of 0.3 μM HMGB1. At the time points of 0, 6, and 12 h after incubation, Laemmlli’s sample buffer was added, and samples were boiled at 100°C for 5 min to stop Aβ degradation. Subsequently, samples were analyzed using the antibody against Aβ (clone 6E10, 1 : 2000; Chemicon) by Western blot analysis, as described previously.

2.6. Immunohistochemical Study Using Human AD Brain Sections. All experiments using human samples were performed in accordance with the guidelines of the ethical committees of Kyoto Pharmaceutical University. Informed consent was obtained from all subjects. For histological examination, frontal cortex tissue from a patient who was clinically and histopathologically diagnosed as human AD (age, 67 years) was used. Neuropathological assessment of AD was conducted in accordance with the criteria of the Consortium to Establish a Registry for Alzheimer’s Disease (CERAD). Dissected tissue blocks were fixed in 10% formalin and transferred to a 15% sucrose solution in 100 mM PB containing 0.1% sodium azide at 4°C. The cryoprotected brain blocks were cut into 20 μm sections on a cryostat, and the collected sections were stored in PBS containing 0.3% Triton X-100 (PBS-T) and 0.1% sodium azide at 4°C until use.

Immunohistochemical study was essentially performed as described previously [34]. Free-floating human brain sections were treated with 0.1% hydrogen peroxide for 30 min to quench endogenous peroxidase activity; they were then incubated with 1% goat serum to block nonspecific binding in PBS. Sections were then incubated with a mouse monoclonal antibody against Aβ40 (1 : 1000; nanoTools, Teningen, Germany) and rabbit polyclonal antibody against Aβ42 (1 : 1000; IBL, Gunma, Japan), a rabbit polyclonal antibody against Aβ40 (1 : 1000; IBL) and mouse monoclonal antibody against human leukemia antigen (HLA)-DR (1 : 50; Dako, Glostrup, Denmark), or a rabbit polyclonal antibody against Aβ42 (1 : 1000; IBL) and mouse monoclonal antibody against HLA-DR (1 : 50; Dako) in PBS-T with 0.1% sodium azide for 4 days at 4°C. The primary antibodies were probed with Alexa Fluor 546-labeled anti-rabbit IgG antibody and Alexa Fluor 488-labeled anti-mouse IgG antibody (each diluted 1 : 500; Molecular Probes). Subsequently, fluorescence was observed using a laser scanning confocal microscope LSM 510 (Carl Zeiss).

2.7. Statistical Evaluation. Results of the densitometric analysis are given as the mean ± standard error of mean. The statistical significance of differences was determined by analysis of variance. Further statistical analysis for post hoc comparisons was conducted using the Bonferroni/Dunn test (StatView, Abacus Concepts, Berkeley, CA).

3. Results

3.1. Binding of HMGB1 with Aβ40. In our previous study, we found that HMGB1 is extracellularly accumulated on Aβ plaques in AD brains and further demonstrated that HMGB1 binds to Aβ42 in in vitro cell-free study [33]. In the present cell-free study, we first examined the binding affinity of HMGB1 for Aβ40. Following incubation of the HMGB1 peptide alone, a 29 kDa band of HMGB1 and its high-molecular-weight aggregates was detected, while an approximately 33 kDa band (arrow in Figure 1(a)), which is believed to be a complex of HMGB1 and Aβ40, appeared as an upper band 6 h after incubation of HMGB1 and Aβ40 peptides (Figure 1(a)). Following incubation with Aβ40 (Figure 1(b)), monomers and oligomers of Aβ40 were the major components present in the absence of HMGB1 at each time point. Predictably, the 33 kDa band, which seemed to be a complex of HMGB1 and Aβ40, was detected by the addition of the HMGB1 peptide (arrow in Figure 1(b)).

To confirm the binding affinity between HMGB1 and Aβ40, we further examined immunoprecipitation using the
3.2. Microglial Aβ Phagocytosis and Effect of Exogenous HMGB1. We previously demonstrated that microglia markedly phagocytose Aβ42 [25], and extracellular HMGB1 inhibits phagocytosis on the cell surface [32, 33]. In the present study, we analyzed the microglial Aβ40 phagocytosis and the effects of extracellular HMGB1 on phagocytosis using laser confocal microscopy (Figure 2). Endogenous HMGB1 was detected in the nuclei of primary cultured rat microglia (Figures 2(a)–2(f), cyan). When treated with the vehicle (Figure 2(a)) or HMGB1 alone (Figure 2(b)), no Aβ immunoreactivity was detected. Consistent with previous studies, in the presence of Aβ42, microglia phagocytosed Aβ42 (Figure 2(c), red), exogenous HMGB1 was colocalized with Aβ42 on the microglial cell surface, and Aβ internalization was inhibited (Figure 2(d), yellow). When treated with Aβ40, the immunoreactivity of Aβ40 was barely detected in the microglial cytoplasm (Figure 2(e), red). Interestingly, in the presence of exogenous HMGB1, small vesicle-like immunoreactivities of Aβ40 (Figure 2(f), red) and HMGB1 (Figure 2(f), green) were markedly increased in the microglial cytoplasm, and parts of them were colocalized with each other (Figure 2(f), yellow).

3.3. Amounts of Aβ40 inside and outside Microglia and Effect of Exogenous HMGB1. Twelve hours after Aβ40 treatment, microglial cell lysate and conditioned medium were collected and subjected to Western blot analysis; semiquantitative analysis was then examined to measure the concentration...
Figure 2: Effect of exogenous HMGB1 on microglial Aβ phagocytosis analyzed by laser confocal microscopy. Rat microglia were incubated with the vehicle (a), HMGB1 (b), Aβ42 (c), Aβ42 and HMGB1 (d), Aβ40 (e), or Aβ40 and HMGB1 (f) for 12 h. Fixed cells were further incubated with the anti-Aβ antibody (red), anti-HMGB1 antibody (green), and Hoechst 33258 (dye for nuclei; blue); they were analyzed using a laser scanning confocal microscope. DIC: differential interference contrast. Scale bar = 20 μm for all panels.

Figure 3: Effect of exogenous HMGB1 on microglial Aβ phagocytosis analyzed by Western blot. Rat microglia were incubated with the vehicle, HMGB1, Aβ40, or Aβ40 and HMGB1 for 12 h. Microglial cell lysate (a) and conditioned medium (b) were then subjected to Western blot analysis using the anti-Aβ antibody, and then the amounts of Aβ40 inside (a) and outside microglia (b) were semiquantitatively measured. * P < 0.05, *** P < 0.001 versus treatment with Aβ40 alone.
3.4. Aβ Degradation with the Microglial Cytosolic Fraction and Effect of Exogenous HMGB1. To confirm whether exogenous HMGB1 inhibits Aβ40 degradation in microglial cytoplasm, we prepared cytosolic fractions from rat microglia and mixed them with Aβ. Degradation of Aβ40 and Aβ42 by microglial cytosol fractions was compared (Figure 4(a)). Aβ40 and Aβ42 were gradually degraded by the addition of the microglial cytosolic fraction in a time-dependent manner. Aβ40 was degraded earlier than Aβ42 (Figure 4(a)). We next examined the effect of exogenous HMGB1 on the Aβ40 degradation induced by the microglial cytosolic fraction (Figure 4(b)). At 6 and 24 h after incubation, the degradation of Aβ40 was significantly delayed by the addition of exogenous HMGB1. Thus, this result suggests that exogenous HMGB1 phagocytosed by microglia inhibits the degradation of Aβ40 in the microglial cytoplasm.

3.5. Accumulation of Aβ40, Aβ42, and Microglia in AD Brains. We further investigated the localization of Aβ40 and Aβ42 in AD brains using specific antibodies (Figures 5(a) and 5(b)) and microglial accumulation on the plaques composed of Aβ40 (Figures 5(c) and 5(d)) and Aβ42 (Figures 5(e) and
Figure 5: Immunohistochemical study of microglial accumulation on Aβ plaques in human AD brains. Free-floating human AD brain sections were incubated with the anti-Aβ40 specific antibody (dark blue) and anti-Aβ42 specific antibody (brown) (a, b), anti-Aβ40 specific antibody (dark blue) and anti-HLA-DR antibody (for microglial staining; brown) (c, d), and anti-Aβ42 specific antibody (dark blue) and anti-HLA-DR antibody (for microglial staining; brown). Arrows and arrow heads show marked and poor microglial accumulations, respectively. (b), (d), and (f) show high-magnification views of squared area in (a), (c), and (e), respectively. Scale bar in (a) equals 400 μm for (a), (c), and (e). Scale bar in (b) equals 100 μm for (b), (d), and (f).

3.6. Accumulation of HMGB1 and Microglia on Aβ40 Plaques in AD Brains. We previously demonstrated that extracellular HMGB1 accumulates on Aβ plaques, as detected using an anti-Aβ antibody that reacts with a broad spectrum of Aβ species [33]. Therefore, in the present study, we investigated the colocalization of extracellular HMGB1 on Aβ40 plaques in AD brains using a specific anti-Aβ40 antibody. Consistent with the immunohistochemical study (Figures 5(c) and 5(d)), microglia (Figure 6(b)) markedly accumulated on Aβ40 plaques (Figure 6(a)) in AD brains (Figure 6(c)). We further demonstrated that extracellular HMGB1

5(f)). The number of Aβ40 plaques (dark blue deposits in Figure 5(a)) was lesser than that of Aβ42 plaques (brown deposits in Figure 5(a)). High-magnification photographs revealed that Aβ40 accumulated on Aβ42 plaques (Figure 5(b)). Regarding microglial accumulations (Figures 5(c)–5(f)), almost all Aβ40 plaques were markedly surrounded by activated microglia (Figure 5(c) and arrows in Figure 5(d)). Although some Aβ42 plaques were markedly accumulated by microglia (Figure 5(e) and arrow in Figure 5(f)), others were moderately or poorly surrounded by microglia (arrowheads in Figure 5(f)).
In studies on familial AD, mutations in the APP genes have been detected, and transgenic mice models carrying these familial AD-linked mutations show enhanced Aβ production in their brains. In particular, transgenic mice carrying the APP mutation display characteristics that closely resemble AD, such as Aβ deposition and memory dysfunction [35, 36], and introduction of the double mutations of PS1/APP exhibits the early onset of these pathologies [37]. Thus, all mutations are involved in Aβ generation, and the accumulation of Aβ in the brain has been strongly suggested to be the primary event driving the pathogenesis of AD. However, familial AD accounts for less than 1% of all AD cases [38]; most cases develop sporadically. Although the etiology of sporadic AD remains much more elusive than that of familial cases, neurological and pathological events in sporadic AD are essentially indistinguishable from those in familial cases. In sporadic AD, a decreased Aβ clearance rate has been reported [39].

One proposed mechanism of Aβ clearance is microglial Aβ phagocytosis [40, 41]. Reports on AD patients treated with Aβ immunization also indicate microglial contribution to Aβ clearance in human AD brains [42, 43]. However, it has been suggested that the ability of microglia to clear Aβ decreases with age and progression of AD pathology [26, 27], and it may, at least in part, account for the dysregulation of Aβ clearance in sporadic AD.

HMGBl inhibits microglial Aβ42 phagocytosis by interfering with Aβ42 internalization [32, 33]. In the present study, we further showed that exogenous HMGBl inhibits the degradation of Aβ40 in rat microglial cytoplasm and subsequently delays Aβ40 clearance. We demonstrated the binding affinities of HMGBl for Aβ40 and Aβ42 [33]. Aβ contains an amino acid sequence (18VFFA21) that has been identified to be essential for aggregation and fibril formation [44]. Interestingly, HMGBl contains a homologous motif (16AFFV19), and this sequence is thought to be critically involved in the interactions of Aβ with HMGBl [33, 45]. Among the many peptidases that have been proposed as Aβ-degrading enzymes [46], insulin-degrading enzyme, cathepsin D, and neprilysin are the principle enzymes involved in microglia-mediated Aβ degradation [25, 47, 48]. Many cleavage sites that are the targets of microglial Aβ-degrading enzymes are located on and in the vicinity of the amino acid sequence (18VFFA21) of Aβ [46]. Therefore, we speculate that the cleavage sites of Aβ are masked by the binding of HMGBl; subsequently, the degradation of Aβ40 may be inhibited in the microglial cytoplasm. In case of Aβ42, Aβ42 itself forms high-molecular-weight fibrils during incubation [33]. Therefore, the binding of HMGBl may stabilize Aβ42 fibril formation, and high-molecular-weight complex of HMGBl and Aβ42 fibril may interrupt the uptake of Aβ42 by microglia. Thus, extracellular HMGBl may serve as a chaperone protein for Aβ and inhibit microglial Aβ clearance by interrupting Aβ40 degradation and Aβ42 internalization by microglia. On the other hand, RAGE, TLR2, and TLR4 are receptors for HMGBl [30, 31]; they are also involved in microglial Aβ phagocytosis [49, 50]. Therefore, there is a possibility that the interactions of HMGBl with these receptors on microglia may be related to the inhibitory events on Aβ.

Consistent with a previous study [13], plaques containing Aβ42 predominantly existed in AD brains, and

Figure 6: Laser confocal microscopic study on the accumulation of HMGBl and microglia on Aβ40 plaques in human AD brains. (a–c) Free-floating human AD brain sections were incubated with the anti-Aβ40 specific antibody ((a); red) and anti-HLA-DR antibody ((b) for microglial staining, green). The merged image is indicated in (c). (d–f) Free-floating human AD brain sections were incubated with the anti-Aβ40 specific antibody ((a); red) and anti-HMGBl antibody ((b); green). The merged image is indicated in (f). Scale bar in (a) equals 50 µm for all panels.
Aβ40 accumulated on parts of Aβ42 plaques. Despite the restricted distribution of Aβ40, almost all plaques containing Aβ40 were markedly surrounded by activated microglia. We previously reported that small oligomers formed by Aβ40 strongly induce rat microglial reactions such as cytokine production [51]. Thus, Aβ40 may play an important role in microglial activation and/or recruitment on Aβ plaques. However, we have found that the level of HMGB1 was significantly increased in AD brains [33], and extracellular HMGB1 accumulated on Aβ plaques. Therefore, in AD brains, microglial degradation of Aβ40 and uptake of Aβ42 may be inhibited by extracellular HMGB1 despite the marked accumulation of reactive microglia on Aβ plaques. Moreover, in the present study, we demonstrated that Aβ40 is more readily degraded by the microglial cytosolic fraction than Aβ42. However, in the presence of exogenous HMGB1, the degradation of Aβ40 by microglia is inhibited, and a lot of Aβ40 granules are existed in the cytoplasm of rat microglia as shown in Figure 2(f). Interestingly, numerous microglia containing Aβ40 granules, but not Aβ42, have also been detected in AD brains [52]. Thus, this event in AD brain is well replicated by the treatment with Aβ40 in the presence of extracellular HMGB1 in primary-cultured rat microglia. Results suggest that our findings in the effect of HMGB1 on rat microglia may reflect on the pathological event induced in AD brain and are expected the critical implication of extracellular HMGB1 in the progression of AD pathologies. In addition, we have postulated that the origin of extracellular HMGB1 is leakage from dead neurons during the progression of AD [32], like ischemic neurodegeneration [53]. Extracellular HMGB1 leaked from dead neurons may then accumulate on Aβ plaques through its binding affinity for Aβ in AD brains.

It has been reported that the released HMGB1 is involved in the pathologies of various inflammatory-related disease [54]. In ischemic stroke [55] and intracerebral hemorrhage especially [56], extracellular HMGB1 is suggested to exacerbate brain insult through the disruption of the blood-brain barrier (BBB), overfacilitation of microglia, and intense production of proinflammatory molecules. These studies also demonstrated that a neutralizing anti-HMGB1 monoclonal antibody and glycyrrhizin which bind to and inhibit cytokine-like activity of HMGB1 attenuate the brain insult induced by transient ischemia and intracerebral hemorrhage in rat, respectively. Therefore, there is a possibility that the neutralizing anti-HMGB1 monoclonal antibody and glycyrrhizin may bind to the extracellular HMGB1 accumulated on the Aβ plaques in the AD brain, cancel the inhibitory effects of HMGB1 on microglial Aβ phagocytosis, and then may provide novel therapeutic options for the AD treatment.

In conclusion, in the present study, we found that HMGB1 extracellularly accumulates on Aβ plaques containing Aβ40 in AD brains. We further demonstrated that HMGB1 has a binding affinity for Aβ40, and exogenous HMGB1 is internalized into rat microglial cytoplasm with Aβ40 and inhibits Aβ40 degradation. Subsequently, exogenous HMGB1 delays Aβ40 clearance in the culture medium. Thus, these results suggest that extracellular HMGB1 attenuates microglial Aβ clearance and is possibly involved in the progression of AD pathology.

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