Promotion in the Clearance of Aggregated Aβ In Vivo Using Amyloid Selective Photo-Oxygenation Technology

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ABSTRACT: Alzheimer’s disease (AD) is characterized by the aggregation and deposition of 2 amyloid proteins: amyloid β peptide (Aβ) and tau protein. Immunotherapies using anti-Aβ antibodies to promote the clearance of aggregated Aβ have recently been highlighted as a promising disease-modifying approach against AD. However, immunotherapy has still some problems, such as low efficiency of delivery into the brain and high costs. We have developed the “amyloid selective photo-oxygenation technology” as a comparable to immunotherapy for amyloids. The photo-oxygenation can artificially attach the oxygen atoms to specific amino acids in amyloid proteins using photocatalyst and light irradiation. We revealed that in vivo photo-oxygenation for living AD model mice reduced the aggregated Aβ in the brain. Moreover, we also showed that microglia were responsible for this promoted clearance of photo-oxygenated Aβ from the brain. These results indicated that our photo-oxygenation technology has the potential as a disease-modifying therapy against AD to promote the degradation of amyloids, resulting in being comparable to immunotherapy. Here, we introduce our technology and its effects in vivo that we showed previously in Ozawa et al., Brain, 2021, as well as a further improvement towards non-invasive in vivo photo-oxygenation described in another publication Nagashima et al., Sci. Adv., 2021, as expanded discussion.

KEYWORDS: Photo-oxygenation, Alzheimer’s disease, amyloid-β, microglia, amyloid

Alzheimer’s Disease
Alzheimer’s disease (AD) is a progressive neurodegenerative disorder associated with cognitive decline. According to the World Alzheimer Report 2021, there are more than 55 million AD patients in the world, and the number of patients is estimated to reach 78 million by 2030.¹ Hence, AD is one of the major social issues worldwide, however, there is no disease-modifying therapy against AD yet. To solve this social issue, the establishment of an effective disease-modifying therapeutic strategy against AD is strongly needed.

In the brains of AD patients, 2 characteristic pathological features are observed: senile plaques and neurofibrillary tangles. Both are composed of proteinous fibrils called “amyloid,” in which proteins are polymerized with an abnormal characteristic structure, cross β-sheet, that is completely different from their native structure. The major component of senile plaques is amyloid β peptide (Aβ), and neurofibrillary tangles are composed of tau protein. Since many studies have shown that the formation of these amyloids proteins is the cause of AD, inhibition of amyloid formation and/or efficient clearance of already deposited amyloids are considered to be disease-modifying therapy against AD. For this purpose, immunotherapy using anti-Aβ or anti-tau antibodies is recently highlighted. As a mechanism, antibodies specifically bind to the amyloid fibrils, leading to recruit microglia, which is one of the immune cell types in the central nervous system, to promote the degradation of amyloids. Aducanumab, which is one of the anti-Aβ antibodies currently in clinical trials, has been granted accelerated approval by the United States Food and Drug Administration.² Moreover, results of clinical trials suggested that the promoted clearance of senile plaques with anti-Aβ antibodies has also led to reducing tau accumulation in the brain. These results suggest that the efficient clearance of amyloids has the potential as a disease-modifying therapeutic strategy against AD. However, antibodies have some problems, such as high doses due to their low permeability to the blood-brain barrier and expensive prices.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported in part by a Grant-in-Aid for Scientific Research (C) (JP19K06683 to Y.H.), a Grant-in-Aid for Challenging Exploratory Research (JP19K22484 to Y.B.), a Grant-in-Aid for Scientific Research (B) (JP19H02622 to Y.H.) and a Grant-in-Aid for Scientific Research (A) (JP19H01015 to T.T. and JP20H04849 to M.K.) from the Japan Society for the Promotion of Science (JSPS), grants from Innovative Research Group by the Strategic International Brain Science Research Promotion Program (Brain/MINDS Beyond) (JP19dm0307030 to Y.H.), AMED-PRIME (JP22gm6410017 to Y.H.) and Strategic International Collaborative Research Program (SICORP) (JP19ym0210058 to Y.S.) from the Japan Agency for Medical Research and Development (AMED).

Commentary
Shuta Ozawa, Yukiko Hori, Yusuke Shimizu, Atsuhiko Taniguchi, Takanobu Suzuki, Wenbo Wang, Yung Wen Chiu, Reiko Koike, Satoshi Yokoshima, Tomohide Fukuyama, Sho Takatori, Youhei Sohma, Motomu Kanai, Taisuke Tomita, Photo-oxygenation by a biocompatible catalyst reduces amyloid-β levels in Alzheimer’s disease mice, Brain, Volume 144, Issue 6, June 2021, Pages 1884–1897, https://doi.org/10.1093/brain/awab058

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Neuroscience Insights

Photo-Oxygenation Promoting of Clearance for Aggregated Aβ

From this perspective, we have been developing “amyloid selective photo-oxygenation technology” as a new disease-modifying therapy that can be comparable to antibodies (Figure 1). This technology is the artificial addition of oxygen atoms to amyloids using a small compound, photocatalyst, that was developed based on structures of amyloid selective fluorescent probes. The photocatalyst recognizes and binds selectively to cross β-sheet structures in amyloids. When a photocatalyst is in a free form, its excited state under light irradiation relaxes to the ground state by chemical bond rotation, resulting in being inactive. On the other hand, when the photocatalyst in an amyloid-binding state is excited by light, the bond rotation is inhibited. Then, the emitted energy activates ambient oxygen to singlet oxygen, resulting in oxygenating amyloids. Hence, photo-oxygenation can show high amyloid-selectivity due to double barriers, amyloid selective binding of photocatalysts, and the necessity of light irradiation. As shown in Ozawa et al., 2021, we clearly showed that our technology could successfully oxygenate not only fibrils formed by synthetic Aβ peptide but also aggregated Aβ obtained from the brain of AD model mice and AD patients. Moreover, we also revealed that photo-oxygenation inhibited the aggregation of Aβ peptides in vitro.

Next, to evaluate the effect of photo-oxygenation in vivo, we directly injected the photocatalyst into the brains of living AppNL-G-F/NL-G-F mice, knocking-in human Aβ with familial AD mutations. This mouse is well known as an AD model mouse in which Aβ is deposited in the brain in an age-dependent manner. Immediately after injection, light with a wavelength of 660 nm was irradiated using LED fiber at the photocatalyst injected site through a guide cannula in the brain, resulting in the success of in vivo photo-oxygenation in the living mouse brain (Figure 1). When this procedure was carried out once a day for a total of 7 times, we found that the amount of Aβ in the brain was reduced to 60%-70% compared with those at the non-photo-oxygenated site (Figure 1). As the Aβ reduction in the brain by administration of anti-Aβ antibodies has also been shown about 50%-70%, this effect of photo-oxygenation was almost comparable.

Because the remarkable decrease of Aβ in the brain even after just 1 week of photo-oxygenation could not be explained by only the inhibitory effect on aggregation, we hypothesized that photo-oxygenated aggregated Aβ would be rapidly cleared from the brain. Then, to examine the metabolism of photo-oxygenated Aβ, we injected pre-formed oxygenated or non-oxygenated aggregated Aβ into the brains of wild-type mice. 24 hours after injection, we found that the remaining amount of oxygenated Aβ in the brain was smaller than those of non-oxygenated Aβ, indicating that the metabolism of oxygenated Aβ is enhanced compared to non-oxygenated Aβ. We next examined the relationship of microglia with the rapid clearance of oxygenated Aβ. Pexidartinib (PLX3397), an inhibitor of the CSF-1 receptor, has been described to remove microglia in the brain. The enhanced metabolism of oxygenated Aβ in wild-type mice that were treated with PLX3397 and depleted microglia in the brain was canceled, suggesting that microglia were responsible for the rapid clearance of oxygenated Aβ (Figure 1). Moreover, the rapid degradation of oxygenated Aβ was inhibited when the microglial cell, MG6, are treated with...
leupeptin, a lysosomal serine/cysteine protease inhibitor. In contrast, no rapid degradation of oxygenated Aβ was observed in the human astrocytoma cell line, H4, strongly supporting the idea that degrading enzymes in the lysosomes of microglia, not other cell types, are responsible for the clearance of photo-oxygenated aggregated Aβ (Figure 1). These results indicated that our amyloid selective photo-oxygenation technology showed 2 effects in vivo; inhibition of Aβ aggregation and promoted clearance of aggregated Aβ via microglia (Figure 1). Therefore, our photo-oxygenation might be comparable to anti-Aβ antibodies as therapeutic technology against AD, reducing toxic aggregated Aβ in the brain.

Future Direction
As described in Ozawa et al., Brain, 2021, we clearly showed the proof of concept of photo-oxygenation as a potential therapeutic strategy against AD. We continue to improve towards clinical use and have developed a new photocatalyst, which has an improved permeability for the blood-brain barrier due to substantially lower molecular weight. This new photocatalyst can oxygenate amyloid selectively as well as the previous one and is also delivered into the brain by non-invasive administration. We have succeeded in non-invasive in vivo photo-oxygenation for living AppNL-G-F/NL-G-F mice by intravenous injection of this photocatalyst and light irradiation of the brain from outside of the skull. Moreover, chronic non-invasive in vivo photo-oxygenation also reduced the amount of Aβ in the brain, indicating a step towards the application of photo-oxygenation technology to humans. Although we still have some issues, such as difficulty in delivering the light energy through the human brain skull, and need further improvement, thus, we believe in the potential of our photo-oxygenation as a therapeutic strategy.

We also have tried the application of photo-oxygenation to other amyloidoses. There are many amyloid proteins other than Aβ, like tau, all of which polymerize into amyloid with a cross β-sheet structure. These amyloids are deposited in several peripheral tissues and the central nervous system, leading to various diseases collectively called amyloidosis. We could also photo-oxygenate amyloid fibrils formed by tau protein, implicating that photo-oxygenation would apply to tau amyloids in tauopathy, such as fronto-temporal dementia and Pick's disease as well as AD. Likewise, our photo-oxygenation is expected to be versatile for various amyloid proteins, for example, α-synuclein, TAR DNA-binding protein 43 kDa, and immunoglobulin, which are related amyloid proteins in Parkinson’s disease, amyotrophic lateral sclerosis, and AL amyloidosis, respectively. In the future, we hope to clarify the effects of photo-oxygenation on other amyloids in vivo.

Acknowledgements
The authors thank Drs. T. C. Saido (Riken Center for Brain Science), T. Saito (Nagoya City University), J. Q. Trojanowski (University of Pennsylvania), and V. M. Y. Lee (University of Pennsylvania) for valuable reagents, materials, and unpublished information. The authors are also grateful to our present and previous laboratory members for helpful discussions. We also thank the patients and families for brain donations.

Author Contributions
YH wrote the draft and revised it. YS, MK, and TT were involved in discussing, drafting, and editing the manuscript. All authors approved the submitted version.

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