Modulation of Neuroinflammation by Low-dose Radiation Therapy in an Animal Model of Alzheimer's Disease

Eun-Jeong Yang

Seoul National University College of Medicine

Hak Jae Kim

Seoul National University Hospital

Jin Ho Kim

Seoul National University Hospital

Jeongmin Yoon

Seoul National University Hospital

Young-Seok Seo

Chungbuk National University Hospital

HYE-SUN KIM (✉ hyisun@snu.ac.kr)

Department of Pharmacology and Biomedical Sciences, Seoul National University, College of Medicine,
103 Daehakro, Jongro-gu, Seoul 03080, Republic of Korea <https://orcid.org/0000-0002-2483-9674>

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Abstract

Background

Recently, several studies have reported that low dose radiation therapy (RT) reduces the release of pro-inflammatory cytokines in inflammatory-degenerative disorders including Alzheimer's disease (AD). AD is the most common cause of dementia, and neuroinflammation is one of the major contributing factors in AD pathogenesis. Thus, low dose RT is expected to be used clinically for treating AD. However, the proper doses, effects, and underlying mechanisms of RT in AD have not been determined. Therefore, in this study, we aimed to determine the appropriate RT dose and schedule for AD treatment, and to investigate the therapeutic effects and mechanisms of low-dose RT in AD.

Methods

We first determined the proper dose and schedule of RT in late stage AD using 8–9-month old 5x familial AD(5xFAD) mice, a well-known animal model of AD, by comparing the effects of a low total dose with a low dose per fraction (LD-LDRT, 5×0.6 Gy) and a low-moderate total dose with a conventional dose per fraction (LMD-CDRT, 5×2 Gy).

Results

LD-LDRT and LMD-CDRT were found to reduce the level of pro-inflammatory cytokines, i.e. CD54, IL-3, CXCL9/10, and CCL2/4 in the hippocampus of 5xFAD mice. Further, increased microgliosis assessed with Iba-1 was significantly reduced by LD-LDRT in the hippocampus of 5xFAD mice. Moreover, LD-LDRT and LMD-CDRT decreased the amyloid plaque burden in 5xFAD mice and attenuated their cognitive impairment; these effects persisted for nearly one month.

Conclusions

The present study showed that LD-LDRT rescues cognitive impairment and prevents accumulation of amyloid plaques by regulating neuroinflammation in the late stage of AD, with an efficacy equivalent to that of LMD-CDRT. Furthermore, it suggests that LD-LDRT may facilitate accessible and convenient treatment in clinical trials compared to LMD-CDRT.

Background

Alzheimer's disease (AD) is the most prevalent cause of dementia (1). An estimated 50 million people worldwide were reported to be living with dementia in 2017 and this number is expected to double every 20 years (1). Despite several efforts in the last decades, no mechanism-based fundamental disease-modifying therapies have been approved for this destructive disease, and currently used therapeutics such as donepezil, rivastigmine, galantamine, tacrine (acetylcholinesterase inhibitors), and memantine (NMDA antagonist) provide only temporary symptomatic relief (2).

Postmortem analysis of the brains of patients with AD has demonstrated that neuroinflammation is involved in AD pathogenesis, together with other factors (3). Various neuroinflammation-related factors, such as interleukin (IL)-1 β , IL-6, and transforming growth factor (TGF)- β , have been found to accumulate around the brain amyloid plaques in AD (4-6). Therefore, numerous studies have investigated the pro-inflammatory and anti-inflammatory cytokine levels in patients with AD. Based on numerous evidences, neuroinflammation has been accepted as an important factor in AD pathogenesis, with studies suggesting various mechanisms including the activation of astrocytes and microglia (7). Further, epidemiologic studies with low incidence of AD in patients with rheumatoid arthritis under long-term treatment with nonsteroidal anti-inflammatory drugs (NSAIDs) indicate that inflammation is involved in AD development (8). However, clinical trials based on NSAIDs such as naproxen and celecoxib in patients with AD have not been successful (9-11). Though neuroinflammation is responsible for AD pathogenesis, there are no effective treatments for regulating neuroinflammation to date. Therefore, alternative treatments are needed to target neuroinflammation in AD.

Radiation therapy (RT) is one of the numerous treatments known to have an anti-inflammatory effect. Interestingly, some countries such as Germany extensively use a low total dose RT with low-dose per fraction (total 3–6 Gy, single dose < 1 Gy) for treating inflammatory-degenerative disorders (12). The interrelationship between RT and the immune system exhibits dichotomous characteristics and is highly dependent on the dose/quality of radiation. A high total dose with conventional-dose RT (total 50–70 Gy, single dose 1.8 Gy) that is generally used in cancer treatment, has the effect of exacerbating inflammation (13), whereas low-dose RT has been reported to control various inflammatory processes and clearly shows anti-inflammatory properties (14). Based on these results, the German Society of Radiation Oncology recommends single fraction doses of 0.5–1.0 Gy and total doses of 3.0–6.0 Gy/series for low-dose RT in painful degenerative skeletal disorders (12).

Surprisingly, patients with AD have been reported to have partially restored cognition, speech, memory, movement and appetite after several brain CT scan exposures (15). Recently, several preclinical studies have revealed a significant reduction of amyloid plaque burden and/or tau staining in mouse models of AD with relatively low-moderate total doses of 9–10 Gy in 5 fractions (16-19). The RT dose in these studies was a relatively low total dose of 10 Gy, which was lower than the 60 Gy generally used in cancer treatment, but the dose per fraction remained the same as the conventional dose RT (1.8–2 Gy). As in the studies on inflammatory-degenerative disorders, it is unclear whether AD shows anti-inflammatory effects and disease improvement by exposure to a low total dose with low single dose RT. Therefore, we aimed to determine the appropriate RT dose and schedule for AD treatment as well as the therapeutic effects and mechanisms of action for low-dose RT in AD.

Methods

Antibodies and reagents

TRIzol reagent was obtained from Qiagen (Hilden, Germany). The 6E10 antibody was purchased from Covance (Princeton, NJ, USA). PSD-95, TO-PRO-3, and Alexa Fluor® 488 goat anti-mouse IgG were obtained from Thermo Scientific (Waltham, MA, USA). Glial fibrillary acidic protein (GFAP) antibody was purchased from DAKO (Glostrup, Denmark). Ionized calcium-binding adapter molecule 1 (Iba-1) antibody was obtained from Novus Biologicals (Littleton, CO, USA). Neuronal nuclei (NeuN) antibody was purchased from Merck Millipore (Darmstadt, Germany).

Animals

Animal treatment and maintenance were performed and approved as per the Animal Care and Use Guidelines of Seoul National University, Seoul, Korea (SNU-181005-1-2). The 5xFAD mouse is a transgenic mouse with 5 mutations (Swedish; K670N/M671L, Florida; I716V, London; V717I in human APP and M146L, L286V in human PS1). 5xFAD mice were purchased from Jackson Laboratories and were maintained by crossing hemizygous transgenic mice with B6SJL F1 mice. Transgenic mice were identified by PCR, and non-transgenic littermates were used as controls.

Radiation therapy

Mice were divided into the sham group without RT, low total dose with low dose per fraction (LD-LDRT) group receiving 3 Gy (5 x 0.6 Gy) radiation, and low-moderate total dose with conventional dose per fraction (LMD-CDRT) group receiving 10 Gy (5 x 2 Gy) radiation. Mice from the RT groups received whole-brain irradiation with 6 MeV electrons at 100 cm SSD every other day for 10 days (Clinac 21EX®, Varian, Palo Alto, CA). A custom made cerrobend block (field size: 20 cm × 8 cm) was used to shield all tissues except the brain from the treatment field. Mice were anesthetized during the RT process using Zoletil and Rompum. For quality assurance of RT, tissue-equivalent phantoms mimicking both the material properties and anatomical shape of real mice were manufactured by 3D printing and a dosimetric analysis was performed. Behavioral tests were performed a week before RT and every week after RT for 4 weeks. Mice were sacrificed for histological and biochemical analyses after completing the behavior tests (Fig. 1).

Spontaneous Y-maze test

The Y-maze consisted of 3 equal arms (40 × 15 × 9 cm) and was constructed using black acrylic plastic. The test was performed at Seoul National University. All mice were placed at the center of the Y-maze at the start of the trial following 20 min of habitation in the room. Mice were allowed to freely explore the three arms for 8 min. All movements were recorded using a video camera and were analyzed to determine the alternation ratio by evaluating the number of arm entries, in triplicate.

Immunohistochemistry

Brains were fixed with 4% paraformaldehyde for 24h and cut into 30 µm-thick sections, which were then incubated overnight with the 6E10 antibody (1:500) at 4°C. The sections were then washed with PBS and

incubated with the secondary antibody (Alexa Fluor® 488 goat anti-mouse IgG, 1:200). The sections were then incubated with TO-PRO-3 (1:1000) for 30 min at room temperature. All samples were mounted on microscope slides in mounting medium. Confocal microscopic observation was performed using the LSM 510 (Jena, Deutschland, Germany) and all images are showed as combined confocal z-stacks. The sizes of the amyloid plaques were measured using Image-J and the plaques were then classified into 3 categories.

Western blot

Hippocampal extract samples (20-40 µg) were separated on 8–10% sodium dodecyl sulphate-polyacrylamide electrophoresis gels and transferred to a nitrocellulose membrane. The membranes were then blocked for 1 h at room temperature in 5% skim milk, followed by overnight incubation at 4°C with the following primary antibodies: anti- Postsynaptic density protein 95 (PSD-95, 1:1000), anti-NeuN (1:1000), anti-GFAP (1:1000), anti-Iba-1 (1:1000), and anti-GAPDH (1:2000). Next, the blots were incubated with the secondary antibody conjugated with horseradish peroxidase for 1 h at room temperature. The resulting bands were detected by enhanced chemiluminescence (Young-in Frontier, Seoul, Korea).

Cytokine array

A Proteome Profiler Array (R&D system, MN, USA) was performed as described previously (20). Briefly, the cerebral cortex was homogenized with PBS and Triton X-100. The resulting lysate (150 µg) was used for the cytokine array to detect 40 mouse cytokines or chemokines.

RT-qPCR

Total RNA from the hippocampus was isolated using TRIzol reagent. First-strand cDNA was synthesized from 500 ng of RNA using AccuPower RocketScript RT Premix (Bioneer, South Korea). RT-qPCR was performed using EvaGreen 2X qPCR Master mix (Applied biological materials, Canada). The thermal cycler consisted of 40 cycles of 95°C for 15s, and 60°C for 30s and gene expression was evaluated using a custom designed primer plate (Bioneer, SM-0000-10). All data were analyzed using CFX manager software (Bio-Rad laboratories, CA, USA) and normalized to the expression of β-actin or GAPDH.

Statistical analysis

All data were represented as the mean ± standard error of the mean. Statistical analysis was performed using an independent t-test or one-way ANOVA with LSD post-hoc comparison (IBM SPSS Statistics 20, IL, USA). A *p*value < 0.05 was considered statistically significant.

Results

Low-dose RT reduced pro-inflammatory cytokine expression and decreased microgliosis in the hippocampus of 5xFAD mice

To determine the effects of low-dose RT on microgliosis the expression of Iba-1, a marker for reactive microglia, was evaluated in the hippocampus from wild type (WT) and 5xFAD mice. The schematic diagram of the study is briefly shown in Fig. 1. As shown in Fig. 2a, 5xFAD-Sham mice showed significantly increased protein levels of Iba-1 compared with that in WT-sham mice (5xFAD-Sham, 2.32 ± 0.16 , $n = 9$; WT-Sham, 1.00 ± 0.15 , $n = 8$). The enhanced Iba-1 expression in the hippocampus was significantly decreased by LMD-CDRT (1.62 ± 0.19 , $n = 8$), and 5xFAD-LD-LDRT mice (2.15 ± 0.18 , $n = 8$) showed only a decreasing tendency compared to 5xFAD-sham mice (Fig. 2a), but statistical significance was not observed.

To determine whether low-dose RT affects the polarization of microglia, the mRNA expression levels of pro-inflammatory and anti-inflammatory cytokines were analyzed. 5xFAD-sham mice showed a significant increase in IL-6 (2.83 ± 0.70), CCL6 (3.95 ± 0.54), and IL-1 β (1.49 ± 0.10) mRNA expression compared with that in WT-sham mice (Fig. 2b-e, IL-6, 1.00 ± 0.08 ; CCL6, 1.00 ± 0.18 ; C IL-1 β , 1.00 ± 0.16). The elevated IL-6 (1.30 ± 0.19) and CCL6 (2.78 ± 0.37) mRNA expression was significantly attenuated in 5xFAD-LD-LDRT (Fig 2b, c). LMD-CDRT also ameliorated the mRNA expression of CCL6 (1.10 ± 0.21) and IL-1 β (0.36 ± 0.10) in 5xFAD mice (Fig. 2c, d). However, IL-4 mRNA expression was not significantly different among all mice groups (Fig. 2e). We also determined the cytokine levels using a proteome profiler array. Among 40 cytokines, we identified 8 cytokines that were significantly increased (CD54, 1.97 ± 0.05 ; CCL2, 1.63 ± 0.29 ; CCL4, 1.46 ± 0.21) or showed an increasing tendency (IL-3, 1.24 ± 0.09 ; IL-16, 1.32 ± 0.18 ; CXCL9, 1.09 ± 0.13 ; CXCL10, 1.24 ± 0.17 ; and CXCL11, 1.25 ± 0.15) in 5xFAD-Sham relative to WT-sham (Fig 3b). After LMD-CDRT, the release of pro-inflammatory cytokines such as IL-3/16 (IL-3, 0.91 ± 0.09 ; IL-16, 0.79 ± 0.10), CCL2/4 (CCL2, 1.00 ± 0.09 ; CCL4, 0.58 ± 0.10), and CXCL9 (1.00 ± 0.09) in 5xFAD mice was significantly reduced (Fig. 3a, b). More interestingly, LD-LDRT diminished the production of inflammatory cytokines such as CD54 (1.35 ± 0.25), IL-3 (1.24 ± 0.09), CXCL9/10 (CXCL9, 0.73 ± 0.14 ; CXCL10, 0.90 ± 0.14), CCL2/4 (CCL2, 0.94 ± 0.09 ; and CCL4, 0.85 ± 0.13) in 5xFAD mice (Fig. 3a, b). Overall, these results indicate that LD-LDRT and LMD-CDRT attenuated microgliosis and reduced the level of pro-inflammatory cytokines, but not anti-inflammatory cytokines.

Low-dose RT reduced mature amyloid plaques, but did not affect the number of amyloid plaques in the hippocampi of 5xFAD mice

Because low-dose RT reduced the release of pro-inflammatory cytokines in 5xFAD mice, we speculated that low-dose RT may attenuate accumulation of amyloid plaques by inhibiting microglial activation. The number and size of amyloid plaques was measured after 6E10 staining to identify the effect of low-dose RT on amyloid plaque burden (Fig. 4a). The number of amyloid plaques was not significantly different among all mice groups (Fig. 4b). However, the area of the amyloid plaques in the hippocampus of 5xFAD mice was significantly decreased by LD-LDRT and LMD-CDRT (Fig. 4c; Sham, $0.0234 \pm 1.20e^{-3}$; LD-LDRT, $0.0199 \pm 9.21e^{-4}$; LMD-CDRT, $0.0196 \pm 1.0425e^{-3}$). Amyloid plaques have been previously classified into different types by area (21). Therefore, we classified the amyloid plaques in the hippocampus based on their areas size and found that the number of amyloid plaques larger than 100 nm^2 were significantly reduced by approximately 12% with LD-LDRT and LMD-CDRT (Fig. 4d; Sham, 30.85 ± 1.82 ; LD-LDRT,

27.22 ± 0.60; LMD-CDRT 25.64 ± 0.39). These data demonstrate that LD-LDRT and LMD-CDRT diminished the mature amyloid plaques in the hippocampus of 5xFAD mice.

Low-dose RT rescued the cognitive impairments in 5xFAD mice

Accumulation of amyloid plaques, a major neuropathological characteristic, together with neurofibrillary tangles, contributes to cognitive impairment, which is one of the major symptoms in AD (22, 23). To confirm the impact of RT on cognitive impairment, a spontaneous Y-maze test was performed before and after low-dose RT, to assess cognitive function and to determine a spontaneous ratio indicating spatial memory (Fig. 5a). Before low-dose RT, the spontaneous alternation ratio in 5xFAD-Sham (0.60 ± 0.02), LD-LDRT (0.54 ± 0.03), and LMD-CDRT (0.51 ± 0.18), mice were compared to that in WT-Sham (Fig. 5b, 0.70 ± 0.03). Surprisingly, cognitive impairment was recovered by LD-LDRT (1st week, 0.62 ± 0.05; 2nd week, 0.60 ± 0.02, 3rd week, 0.64 ± 0.06; 4th week, 0.60 ± 0.04) and LMD-CDRT (1st week, 0.62 ± 0.05; 2nd week, 0.60 ± 0.02, 3rd week, 0.58 ± 0.03; 4th week, 0.55 ± 0.03) and its effect lasted for 4 weeks (Fig. 5c). Both body and brain weight showed no significant differences between Sham and RT group mice (Fig. 5d and 5e). These results suggest that LD-LDRT and LMD-CDRT attenuates cognitive impairment in AD without significant adverse effects.

Low-dose RT did not affect neuronal death or synaptic plasticity-related proteins, but reduced reactive astrocytes in 5xFAD mice

Neuronal cell death and impaired synaptic plasticity have been associated with the accumulation of amyloid plaques or cognitive impairment in AD (24, 25). To examine whether low-dose RT could protect neuronal cell death or change synaptic plasticity by decreasing amyloid plaque burden, we analyzed neuronal loss and synaptic density in the hippocampus by examining the protein level of NeuN and PSD-95, which are markers for neurons and postsynapses, respectively. The protein level of NeuN (1.02 ± 0.08) and PSD-95 (0.43 ± 0.08) was significantly reduced in 5xFAD-Sham mice. The expression of both NeuN (LD-LDRT, 0.93 ± 0.04; LMD-CDRT 0.85 ± 0.09) and PSD-95 (LD-LDRT, 0.57 ± 0.10; LMD-CDRT 0.38 ± 0.08) was not affected by low-dose RT (Fig. 6a). Taken together, low-dose RT did not affect neuronal death or impaired synaptic plasticity even though it reduced the amyloid plaque burden. Next, we examined the astrocyte reactivity after low-dose RT in 5xFAD mice. Reactive astrocytes that are close to amyloid plaques are associated with the development and progression of AD (26). The increase in GFAP expression observed in 5xFAD-sham mice (1.92 ± 0.21) was also attenuated in 5xFAD-LD-LDRT (0.95 ± 0.17), but not in 5xFAD-LMD-CDRT (Fig. 6b).

Discussion

Despite the increasing prevalence and incidence rates of AD, there are no efficient disease-modifying therapies that have been successful in clinical trials (2). New promising drugs for AD are being intensively developed by many researchers and pharmaceutical companies, but these still have several limitations. First, drugs need to reach the brain after systemic administration via the blood brain barrier (BBB). The

BBB is formed by tight junctions of microvascular endothelial cells, pericytes and perivascular astrocytes and prevents the brain from toxins and pathogens (27). Drug delivery via the BBB is still challenging in AD with many central nervous system (CNS) disorders. Second, the majority of developing drugs target amyloid plaques or tau, the two hallmarks of AD. However, over the decades, numerous clinical trials have failed as they were performed in the late stage of AD (28). Therefore, early diagnosis of the disease and new perspectives in overcoming the existing hurdles is very important for AD treatment and therapeutics.

RT is one of the most commonly used treatment modalities against various kinds of cancers. Recently, several studies have reported that low-dose RT attenuates inflammation in painful-degenerative diseases and in AD (12, 14, 17-19). In this study, we investigated the therapeutic effects of low-dose RT in the late stage of AD using 5xFAD mice.

Microglia are the only resident immune cells in the CNS and play a critical role in neuroinflammation (29, 30). Neuroinflammation is reported to contribute to AD pathogenesis and inflammatory responses are implicated in microglial activation (31, 32). Therefore, regulation of reactive microglia can be a potential therapeutic target in AD. To elucidate whether RT prevents neuroinflammation by inhibiting microglial activation, we examined the protein expression of Iba-1, a well-known microglial marker. Iba-1 expression was significantly decreased in the hippocampus of 5xFAD mice by LMD-CDRT, but not by LD-LDRT (Fig 2). Here, the lack of significantly reduced microglial activation in the 5xFAD-LD-LDRT group cannot be regarded as a negative result.

Microglia are divided into M1 or M2 phenotypes (33). M1-type microglia produce pro-inflammatory cytokines such as IL-4, IL-13 whereas M2-type microglia release anti-inflammatory cytokines such as IL-6, IL-1 β (34, 35). Especially, M2 type microglia are known to increase in the early stage of AD, whereas M1 type microglia are increased in the late stage of AD (32, 36). To clarify the type of microglia existing after RT, we measured the levels of several cytokines through a cytokine array. After LD-LDRT or LMD-CDRT, the level of pro-inflammatory cytokines such as IL-3, CXCL 9/10, and CCL2/4 was significantly reduced (Fig 3). The mRNA level of pro-inflammatory cytokines was also decreased after RT (Fig 2). Taken together, LD-LDRT regulates neuroinflammation by decreasing pro-inflammatory cytokines and has effects similar to LMD-CDRT in the AD mouse model. This suggests that low-dose RT may shift the M1/M2 ratio in the hippocampus of 5xFAD mice. This mechanism was recently confirmed by Kim et al. in a study showing that the M2 marker CD206 was significantly increased in lipopolysaccharide (LPS)- and LMD-CDRT-treated BV-2 microglial cells compared with that in LPS- and sham-treated BV-2 cells; the effect of LMD-CDRT on M2 polarization was confirmed by increased TREM2 expression in LPS-induced BV2 cells (37). Amyloid plaques are reported to include reactive astrocytes and microglia, and these glial cells are recruited to clear the amyloid plaques. In addition, neuroinflammation caused by reactive astrocytes and microglia induces the production of amyloid beta peptide (A β) and amyloid plaques (38). Thus, there is a vicious cycle between neuroinflammation and accumulation of amyloid plaques, and it may be important to break this vicious cycle in AD. Our results suggested that low-dose RT affects the accumulation of amyloid plaques by regulating neuroinflammation in AD.

Accumulation of amyloid plaques is considered a major neuropathological hallmark of AD (39). Neuroinflammation has been suggested to increase pro-inflammatory cytokines, induce A β production, and the aggregation and accumulation of amyloid plaques (40). Next, we investigated the relationship between RT and amyloid plaque accumulation. Few studies have reported that RT affects A β deposition (16-18). However, these studies examined the therapeutics effects of RT in AD after LMD-CDRT, and not LD-LDRT. In the present study, we confirmed that low-dose RT did not change the number of amyloid plaques in the hippocampus. However, the amyloid plaque burden or the area of amyloid plaques was significantly decreased in 5xFAD-LD-LDRT and 5xFAD-LMD-CDRT (Fig 4). Further, reduction was observed in the large-sized amyloid plaques. This suggested that low-dose RT prevents amyloid plaque aggregation.

Cognitive impairment, a major symptom of AD is observed from 6 months of age in 5xFAD mice (41). In this study, cognitive function was confirmed before and after low-dose radiation exposure (LD-LDRT, LMD-CDRT) in an AD mouse model. Both 5xFAD-LD-LDRT and 5xFAD-LMD-CDRT mice showed rescue in cognitive impairment and this attenuating effect lasted for 4 weeks (Fig. 5e). To remove the possibility of radiation toxicity, we also checked the body and brain weight. Neither LD-LDRT nor LMD-CDRT resulted in altered body or brain weight (Fig. 5b, c). This suggests that low-dose RT has therapeutic effectiveness in late stage AD and that it has a long-term effect without any serious side effects.

Next, we investigated the molecular changes in neurons and non-neuronal cells such as astrocytes. Low-dose RT did not affect neuronal death or change synaptic plasticity-related proteins in neurons (Fig. 6A). Increasing astrocyte reactivity in the brains has been observed in the brain in AD (26). Recently, several studies reported that inhibition of reactive astrocytes is an important factor to attenuate cognitive impairments (42, 43). Our study demonstrated that LD-LDRT decreased the expression of GFAP, a reactive astrocyte marker, in the hippocampus of 5xFAD mice (Fig. 6B). Recently, we reported intriguing results that astrocytes reside in a new state called memory-induction state and that astrocytes in this state are actively involved in memory formation. Reactive astrocytes exert detrimental effects of cognitive dysfunction in AD, as reactive astrocytes cannot change into the memory-induction state during memory induction (44, 45). Further studies are thus needed to investigate whether attenuation in the increase of reactive astrocytes is also involved in alleviating cognitive dysfunction in the AD animal model after LD-RT.

Low-dose RT can be administered by reducing the total dose and/or reducing single fraction doses. In previous studies on painful-degenerative disease, low-dose RT was performed by reducing both the total radiation dose and the single fraction dose (LD-LDRT)(12, 14); however, only the total radiation dose was reduced without reducing the single fraction dose (LMD-CDRT) in previous studies on low-dose RT for AD (16-19). To the best of our knowledge, the present study is the first study to apply LD-LDRT to an AD mouse model. Both LD-LDRT and LMD-CDRT were effective in improving cognitive impairment and neuroinflammation in the 5xFAD AD mouse model. If there is no difference in the treatment efficacy, it would be reasonable that the radiation dose is kept as low as possible. Currently, clinical studies are underway to treat AD using RT; most of these studies are using LMD-CDRT (ClinicalTrials.gov Identifier:

NCT02359864; NCT02769000; NCT03352258; and NCT04203121). Based on the results of present study, it may be possible to conduct a clinical trial to treat AD with LD-LDRT, which is less than half of the RT dose used in these preceding clinical trials using LMD-CDRT.

Conclusions

LD-LDRT (3 Gy in 5 fractions) rescues cognitive impairment and prevent accumulation of amyloid plaques in a mouse model by regulating neuroinflammation in the late stage of AD; its effectiveness was equivalent to that of LMD-CDRT (10 Gy in 5 fractions). LD-LDRT may thus make treatment accessible and convenient in clinical trials compared to LMD-CDRT. Based on those results, it would be necessary to evaluate proper RT dose and time, including safety, in clinical trial. Overall, LD-LDRT may be a promising treatment for AD, and further research is needed to confirm the various underlying mechanisms.

Abbreviations

5xFAD: 5x familial AD

AD: Alzheimer's diseases

A β : Amyloid beta peptide

BBB : Blood brain barrier

CNS : Central nervous system

GFAP: Glial fibrillary acidic protein

Iba-1: Ionized calcium-binding adapter molecule 1

IL: Interleukin

LD-LDRT: Low total dose with a low dose per fraction

LMD-CDRT: Low-moderate total dose with a conventional dose per fraction

LPS: Lipopolysaccharide

NeuN: Neuronal nuclei

NSAIDs : Nonsteroidal anti-inflammatory drugs

PSD-95: Postsynaptic density protein 95

RT: Radiation therapy

TGF : Transforming growth factor

WT : Wild type

Declarations

Ethical Approval and Consent to participate

All animal treatment and maintenance were performed and approved as per the Animal Care and Use Guidelines of Seoul National University, Seoul, Korea (SNU-181005-1-2).

Consent for publication

Not applicable

Availability of supporting data

All data analyzed during this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare no competing interests.

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Authors' information

Affiliations

Department of Pharmacology and Biomedical Sciences, Seoul National University, College of Medicine, 103 Daehakro, Jongro-gu, Seoul 03080, Republic of Korea

Eun-Jeong Yang, Hye-Sun Kim

Neuroscience Research Center, Seoul National University, College of Medicine, 103 Daehakro, Jongro-gu, Seoul 03080, Republic of Korea

Eun-Jeong Yang, Hye-Sun Kim

Department of Radiation Oncology, Seoul National University Hospital, 103 Daehakro, Jongro-gu, Seoul 03080, Republic of Korea

Hak Jae Kim, Jin Ho Kim

Department of Radiation Oncology, Seoul National University College of Medicine & Hospital, 103 Daehakro, Jongro-gu, Seoul 03080, Republic of Korea

Hak Jae Kim

Cancer Research Institute, Seoul National University, Seoul, Republic of Korea

Hak Jae Kim

Department of Radiation Oncology, Sheikh Khalifa Specialty Hospital, Ras Al Khaimah, United Arab Emirates

Jeongmin Yoon

Department of Global Operations and Business Development, Seoul National University Hospital, Seoul, Republic of Korea

Jeongmin Yoon

Department of Radiation Oncology, Chungbuk National University Hospital, Seowon-gu, Cheongju 28644, Korea

Young-Seok Seo

Seoul National University Bundang Hospital, Seongnam, Sunghnam, Bundang-Gu 13620, Republic of Korea

Hye-Sun Kim

Corresponding author

Correspondence to Young-Seok Seo and Hye-Sun Kim

Authors' contributions

Conceptualization, and Y.S.S. and H.S.K.; Designed methodology, H.J.K., J.H.K., J.Y. and E.J.Y.; Conducted the investigation, E.J.Y. Manuscript writing and editing, E.J.Y., Y.S.S. and H.S.K. All authors read and approved the final manuscript.

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Figures

Fig 1.

Figure 1

A schematic summary of the experimental procedure. 8–9-month old mice were treated with low dose radiation (the LD-LDRT group was exposed to 3 Gy (5 × 0.6 Gy) radiation and the LMD-CDRT group was exposed to 10 Gy (5 × 2 Gy) for 10 days. The behavior test was performed a week before LT and every week after LT for 4 weeks. Histological and biochemical analyses were conducted after completing the behavior test.

Fig 2.

Figure 2

Effect of low-dose RT on microgliosis and cytokine mRNA expression in the hippocampus or cerebral cortex of 5xFAD mice. (a) Representative blot images (upper) and bar graph representing mean

concentration of Iba-1 protein expression (lower). (b-e) Bar graphs displaying the changes in the mRNA levels of pro-inflammatory cytokines ((b) IL-6, (c) CCL6, (d) IL-1 β) or an anti-inflammatory cytokine ((e) IL-4) by low-dose RT. The statistical analyses were performed by one-way ANOVA, and the data are presented as the means $\pm$ SEM (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. WT-sham, $n = 3-6$ /group).

Fig 3.

Figure 3

Modulatory effect of low-dose RT on inflammatory cytokine production in the cerebral cortex of 5xFAD mice. (a) Representative image of the cytokine array data in all groups. (b) Bar graphs showing the release of pro-inflammatory cytokines (sICAM, IL-3, IL-16, CXCL9/10/11, CCL2/4) following low-dose RT among 40 cytokines. The mean densities of each spot were measured using reference spots. Statistical analyses were performed by one-way ANOVA, and the data are presented as the means $\pm$ SEM (* $p < 0.05$ vs. WT-Sham, # $p < 0.05$, ## $p < 0.01$, vs. 5xFAD-Sham, $n = 3$ /group).

Fig 4.

Figure 4

Inhibitory effect of low-dose RT on amyloid plaque accumulation in the hippocampus of 5xFAD mice. (a) Representative confocal images of amyloid plaques stained using the 6E10 antibody in all groups. Scale bar denotes 100 µm (b-d) Bar graphs illustrating (b) the number of amyloid plaques, (c) the size of amyloid plaques, and (d) the number of amyloid plaques classified by size. All quantifications were carried out based on images. Statistical analyses were performed by one-way ANOVA, and the data are presented as the means ± SEM (* p < 0.05, ** p < 0.01, vs. WT-Sham, n = 7–9/group).

Fig 5.

Figure 5

Protective effect of low-dose RT on cognitive impairment in 5xFAD mice. (a) The experimental timeline is shown. All mice were tested for hippocampal-dependent spatial memory (Y-maze test) before and after exposure to 5 fractions of low-dose RT (LD-LDRT and LMD-CDRT). (b) Bar graphs showing the spontaneous alternation ratio in all mice groups before low-dose RT. (c) Bar graphs illustrating changes in the spontaneous alternation ratio in 5xFAD mice after low-dose RT for 4 weeks. (d-e) Bar graphs displaying the (d) body weight and (e) brain weight in all mice groups after low-dose RT. The statistical analyses were performed by one-way ANOVA, and the data are presented as the means $\pm$ SEM (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. WT-Sham, # $p < 0.05$, ## $p < 0.01$, vs. 5xFAD-Sham, $n = 7-9$ /group).

Fig 6.

Figure 6

Effect of low-dose RT on synaptic plasticity and astrogliosis in the hippocampus of 5xFAD mice. (a) Representative blot images (upper) and bar graphs representing the mean protein expression of PSD-95 (lower), following low-dose RT. (b) Representative blot images (upper) and a bar graph illustrating the mean protein expression of GFAP (lower) following low-dose RT. Statistical analyses were performed by one-way ANOVA, and the data are presented as the means $\pm$ SEM (* p < 0.05, ** p < 0.01, *** p < 0.001 vs. WT-sham, n = 7–9/group).