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Irradiation in Alzheimer's Disease

Cranial irradiation significantly reduces beta amyloid plaques in the brain and improves cognition in a murine model of Alzheimer's Disease (AD)

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ABSTRACT

Background and purpose: To investigate if cranial X-irradiation reduces amyloid-β (Aβ) plaques and influences cognitive function in a transgenic mouse model of AD.

Methods and materials: B6.Cg-Tg (APPswePSEN1dE9)85Dbo/J AD-prone mice were given cranial X-irradiation. The number of Aβ plaques, along with expression of AD specific genes (84 genes: Mouse Alzheimer's Disease RT^2 Profiler™), radiation-associated cytokines (Milliplex® MAP Mouse Cytokine Chemokine Immunoassay) and immunohistochemistry (IL10, IL-1β, Iba1 CD45) was assessed. Behavioral testing was performed to relate changes in Aβ burden to cognitive function using a Morris water-maze task.

Results: Single X-ray doses reduced the number ($p = 0.002$) and size ($p = 0.01$) of Aβ plaques. Low-dose fractionation produced greater 50.6% (1 Gy × 10), 72% (2 Gy × 5) and 78% (2 Gy × 10) reductions. Irradiation was associated with gene (Pkp4, 1.5-fold, $p = 0.004$) and proteomic (MIP-2, 8-fold, $p = 0.0024$) changes at 24–48 h. Microglia increased at 4 weeks post-irradiation ($p = 0.001$). The reduction in Aβ burden (2 Gy × 5) was associated with cognitive improvement ($p = 0.012$).

Conclusion: This is the first report that a clinically relevant course of external beam irradiation (2 Gy × 5) produces a significant reduction in AD-associated amyloid-β plaques with a subsequent improvement in cognitive function. However, longer-term studies are needed to define the precise underlying mechanism and longevity of this response.

Alzheimer's Disease (AD) is the most common form of dementia among the elderly and affects over five million individuals in the United States [25]. The major pathological hallmarks of AD are the accumulation of senile amyloid-β plaques [28] and the development of insoluble neurofibrillary tangles of Tau protein [17]. The abnormal processing and accumulation of amyloid-β initiate a cascade of events culminating in neuronal damage leading to progressive clinical dementia [8,30]. In late-onset AD, the amount of amyloid-β that accumulates can be ~100–200-fold higher than normal [18]. The most recognized hypothesis proposes that AD can be attributed to an imbalance between the production and clearance of amyloid-β [11], but recent evidence also suggests that inflammation may be an important third component which contributes to disease progression once neurodegeneration has begun [12].

Eliminating amyloid-β has been demonstrated as a beneficial treatment strategy for AD patients, and anti-amyloid therapies remain a rational approach for preventing or delaying AD [23]. Amyloid-β is produced by the proteolytic cleavage of amyloid-β precursor protein (AβPP) by β- and γ-secretases, and many novel pharmaceuticals are being developed to prevent the initial cleavage of APP [3,16]. The blood–brain barrier (BBB) has limited or thwarted the success of many of these agents either by preventing the drugs from initially crossing into the brain, or by ensuring the rapid removal of those drugs that can cross the BBB [15]. Delivery of an anti-amyloid therapy that is independent of the blood–brain barrier would be a promising new approach.

One strategy not investigated previously for the treatment or elimination of amyloid-β plaques associated with AD is ionizing

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radiation therapy (RT). Radiation therapy has been shown previously to reduce amyloid-like deposits in extra-cranial disease sites [19,21,24].

Methods

Murine model

Male B6.Cg-Tg (APPswePSEN1dE9)85Dbo/J (005864) mice were purchased from The Jackson Laboratory (Bar Harbor, ME) or bred in-house from commercially purchased breeding stock. These double transgenic mice express a chimeric mouse/human amyloid precursor protein (Mo/HuAPP695swe) and a mutant human presenilin 1 (PS1-dE9), both directed to CNS neurons. Both mutations are associated with early-onset Alzheimer's Disease. Animals were maintained using standard husbandry techniques in accordance with the approved research protocol and Institutional Animal Care and Use Committee guidelines.

Irradiation procedure

At 30 weeks of age, animals were randomized into groups (n = 3–6 per group) and the right half of the brain X-irradiated at room temperature with either a single dose of 5 Gy, 10 Gy or 15 Gy (160 kVp Faxitron X-ray machine model 43855F [Cu and Al filters with HVL equivalent to 0.77 mm Cu]) using a dose rate of 0.69 Gy/min, and sacrificed either 2, 4 and 8 weeks later. A second cohort of animals was treated with three different lower-dose schedules 1 Gy × 10, 2 Gy × 5, or 2 Gy × 10. Animals were immobilized using 1–3% isoflurane (balance 100% O_2) and held on 0.4% isoflurane during irradiation to maintain treatment precision. A lead irradiation jig was used to shield all other tissues from the treatment field including the left-side of the brain. After X-irradiation, the animals were recovered and returned to standard housing.

Tissue harvesting

Animals were euthanized 24 h, 2, 4, or 8 weeks post-radiation. The whole brain was harvested. The anterior coronal region was sectioned using a mouse brain mold and immediately placed in microcentrifuge tubes, snap frozen by immersion in liquid nitrogen, and stored at −80 °C until use (described below). Expression assays were performed twice (2 × n = 3 animals; total of n = 6 animals) following manufacturer's guidelines with duplicate measurements per sample. The remainder of the brain was fixed in 10% zinc formalin for 24 h followed by immersion in 70% ethanol, and then subsequently paraffin embedded using standard procedures.

Staining for amyloid-β plaques

Coronal tissue sections (5 μm) were cut and mounted for antibody-specific immunohistochemistry, standard hematoxylin and eosin (H&E) for morphology and Nissl staining to assess neuronal cell density. A minimum of three sections were cut per animal. Beta amyloid plaques were visualized in coronal brain sections at mid-hippocampal level (−1.70 to −1.94 mm Bregma) using the method of Christensen et al. [7] with several modifications. Briefly, slides were washed in tris-buffered saline (TBS) then pretreated in 88% formic acid for 3 min followed by an additional TBS rinse. After treatment with 0.3% H_2O_2 peroxidase block, additional binding sites were blocked using CAS block (Cat. #00-8120, Invitrogen). Sections were incubated with primary antibody (Aβ 6E10, 1:15,000, mouse monoclonal, Cat. #SIG-39320, COVANCE) at room temperature for two hours then subjected to secondary antibody, polymer and DAB according to the *PicTure*™-MAX polymer detection kit (Cat. #87-9683, ZYMED Laboratories). Brain sections were analyzed by light microscopy and the number of amyloid-β plaques compared between the untreated and irradiated halves of the brain.

Quantitation of amyloid-β plaque, immunohistochemistry and Nissl staining

Three stained coronal slices per mouse were analyzed to compare the number and size of beta-amyloid plaques in the irradiated versus untreated sides of the brain. Aβ plaques were counted and analyzed with Image Pro Plus (Media Cybernetics, Rockville, MD) and NIH ImageJ software (NIH, USA). Tissue sections adjacent to those stained with Aβ were individually stained for IL10, (Abcam cat #ab33471), IL-1β (Santa Cruz SX7884), Iba1 (Abcam cat #ab1560) or CD45 for microglia (Abcam cat #ab10558). Staining intensity was assessed from six high power fields per hemi-brain and the percent positive cells determined with NIH ImageJ. The irradiated and unirradiated sides of the brain were compared. For Nissl stained sections, ten randomly selected fields within the neocortex were captured.

Gene and proteomic studies

Mouse Alzheimer's Disease RT^2 Profiler™ PCR (SABiosciences cat #PAMM057A-24, lot #733107735B) was used to assess expression of 84 genes associated with Alzheimer's Disease. RNA extraction was performed using Qiagen RNEasy Mini Kit (cat #74104) and cDNA synthesis using Qiagen RT2 cDNA Synthesis Kit (cat #330401). Milliplex® MAP Mouse Cytokine Chemokine Immunoassay (Millipore cat #MPXMCYTO-70K-12, Lot #2123058) contained G-CSF, GM-CSF, IFNγ, IL-10, IL-1α, IL-1β, IL-4, IL-6, MCP-1, MIP-1α, MIP-2 and VEGF, and expression was measured in brain and circulating in blood. From the immunoassay, Median Fluorescent Intensity (MFI) data using a 5-parameter logistic or spline curve-fitting method was used to calculate cytokine/chemokines concentrations as per manufacturers' guidelines.

Cognitive testing

64-week old male mice were given whole brain irradiation (n = 19) or sham-treated exposures (n = 14) and evaluated 8 weeks later. Spatial learning and memory were assessed in a Morris maze protocol over two 5-day periods, once before treatment (5 days prior to RT) and once after treatment (8 weeks after RT). The mice were trained to locate a platform submerged in a pool of opaque water (22 °C ± 1 °C) in 3 trials/day with a 30-min inter-trial interval over 5 consecutive days. Latency to find the platform was measured.

Statistics

Paired samples statistics (Student's t-test) was performed to compare the number of plaques between the irradiated and shielded sides of the brain and ANOVA to consider differences between the different doses and times post-irradiation. P values of less than 0.05 were considered statistically significant.

Results

Determining the effect of cranial radiation

The number of Aβ plaques in the cortex of 30-week old naïve transgenic animals varied considerably (mean = 82 (SD ± 49), range 48–180; n = 5). Determining the effect of cranial irradiation on Aβ