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A Model for Assessing Cognitive Impairment after Fractionated Whole-Brain Irradiation in Nonhuman Primates

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Abstract

To investigate the effect of fractionated whole-brain irradiation on nonhuman primates, 6–9-year-old male rhesus monkeys were irradiated with 40 Gy delivered as two 5-Gy fractions/week for 4 weeks. Cognitive function was assessed 5 days/week for 4 months prior to fractionated whole-brain irradiation and for 11 months after irradiation using a Delayed-Match-to-Sample (DMS) task at both low and high cognitive loads. Local rates of cerebral glucose metabolism were measured prior to and 9 months after irradiation using [¹⁸F]-2-deoxy-2-fluoro-D-glucose positron emission tomography. Low cognitive load trials did not reveal a significant reduction in performance until 7 months after irradiation; performance then declined progressively. In high cognitive load trials, the initial impairment was observed ~1 month after irradiation. This was followed by a transient recovery period over the next 1–2 months, after which performance declined progressively through 11 months after irradiation. Nine months after irradiation, glucose uptake during the DMS task was decreased in the cuneate and prefrontal cortex and was increased in the cerebellum and thalamus compared with the levels prior to irradiation. Results from this pilot study suggest that the radiation-induced changes in cognition and brain metabolism observed in rhesus monkeys may be similar to those observed in brain tumor patients receiving brain irradiation.

INTRODUCTION

Presently ~200,000 patients/year in the U.S. are treated with fractionated partial- or whole-brain irradiation for primary or metastatic brain tumors (1); ~100,000 survive long enough (>6 months) to experience radiation-induced brain injury, including cognitive impairment. Of these patients, ~10% develop progressive dementia, and up to 90% show some form of higher-order cognitive impairment that adversely impacts their quality of life (2). Although

short-term interventions can modulate radiation-induced cognitive impairment (3), there are no proven successful long-term treatments or effective preventive strategies (4).

Preclinical studies have shown that irradiating the adult rodent brain leads to (1) a marked and persistent reduction in neurogenesis (5), (2) neuroinflammation (6), and (3) chronic, progressive loss of cognitive function (7). These studies have provided valuable insights into the pathogenesis of radiation-induced brain injury. However, differences in brain structure/organization between rodents and humans (8) and the difficulty of measuring higher-order executive functions in rodents decrease the likelihood of successfully translating these results directly to the clinic.

Because of their close evolutionary relationship to humans, nonhuman primates (NHPs) play a unique role in translational science by bridging the gap between laboratory and clinical investigations (9). NHPs usually have cognitive abilities that cannot be assessed in other species (10). Indeed, one of the major features that distinguishes NHPs and humans from other animals is their brain structure, especially the expansion of the cerebral cortex, which allows sophisticated cognitive processing and behavioral flexibility. Thus, in contrast to rodents, NHPs can be used to examine cognitive processes and behavioral tasks similar to those found in humans (11) and to interrogate brain structure and function with the same noninvasive neuroimaging techniques used to study human radiation-induced brain injury (12).

As a prerequisite to developing an NHP model of radiation-induced higher-order cognitive impairment, we performed a pilot study to determine whether irradiating the NHP brain with a total dose of fractionated whole-brain irradiation that should be biologically similar to that delivered to glioma patients would lead to a progressive decline in higher-order cognitive function.

MATERIALS AND METHODS

Animals and Irradiation Procedures

All animal procedures were in accordance with the Guide for Care and Use of Laboratory Animals, followed protocols for environmental enrichment and psychological well-being, and were approved by the Wake Forest University School of Medicine Institutional Animal Care and Use Committee. Wake Forest is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care. Three 6–9-year-old adult male rhesus macaque monkeys (*Macaca mulatta*) weighing 8.0–12.0 kg were entered into the study. They were housed individually in stainless steel cages in temperature- and humidity-controlled rooms with lighting maintained on a 6:00 a.m./6:00 p.m. on-off schedule and were fed a diet of monkey chow supplemented by fresh fruit to maintain daily-monitored body weight.

Fractionated whole-brain irradiation (40 Gy, 8 fractions of 5 Gy, twice/week for 4 weeks) was administered using 6 MV X rays from a LINAC. This fractionated whole-brain irradiation regimen delivers a biologically effective dose [BED (13)] of 106.7 Gy, assuming an α/β ratio of 3 Gy for late radiation-induced brain injury, which is similar to the BED of 100.2 Gy for the conventional fractionated regimen used to treat glioma patients. Dose was specified at the midplane of the brain (nominal depth = 4.5 cm, nominal midplane diameter = 9.0 cm), calculated for an off-axis point centered in the middle of each radiation field, and delivered isocentrically via opposed right and left lateral fields with the central axis of each beam passing through the outer canthus. Cylindrical alloy eye blocks shielded both eyes for each lateral field. The olfactory region, which in the nonhuman primate brain extends anteriorly between the eyes, was also shielded by the eye blocks.

Multi-Item Visual Delayed-Match-to-Sample (DMS) Task

The basic paradigm for the DMS task in the proposed studies has been described previously (14). Briefly, NHPs were placed in primate chairs facing a computer screen on which images were presented. Trials were initiated by placing a movable cursor onto a start-ring image (circle) followed by presentation of a single clip-art Sample image that the animal selected to initiate the variable interval of 1–30 s (Fig. 1). After the delay interval, two to six images were presented randomly in any of nine different screen positions. If the NHP placed the cursor onto the sample image for 1.0 s, it obtained a juice reward from a sipper tube positioned next to its mouth. Placement of the cursor onto any of the non-sample (distracter) images in the Match phase terminated the trial with no reward, and an error was recorded. The screen was then blanked out for 10 s before initiation of the next trial.

Two parameters were varied randomly during each trial: duration of the delay interval (1–30 s) and the number of clip-art images (two to six) that appear in the Match phase. A critical feature of this version of the DMS task was that each individual clip-art image was selected from a set of 5,000 (changed monthly) and was used only once per 70–150-trial session to maintain trial uniqueness and to decrease interference or generalization of stimulus features across trials. It was not possible for the NHPs to learn or anticipate the cognitive load on a given trial since both the duration of delay and the number of distracter images were imposed randomly in different combinations after the NHP responded to the sample image. Because there were only three NHPs in this study and it was unclear how rapidly the radiation-induced changes would occur, the DMS performance of each NHP was measured 5 days/week for 11 months after fractionated whole-brain irradiation and compared to the stable DMS performance on the same parameters during the 4 months immediately prior to irradiation. Changes in mean percentages of correct trials were compared on a month-to-month basis with the unirradiated control levels. Significance levels were set at $P < 0.001$ rather than $P < 0.05$ to account for the small sample size.

Measurement of Local Rates of Cerebral Glucose Metabolism (CMR_{glc})

The CMR_{glc} for each NHP was determined 1 week prior to the start of fractionated whole-brain irradiation and 9 months after n irradiation using blood sampling and positron emission tomography (PET) as described previously (15). After acclimation to the PET scan procedures, baseline DMS task scans were obtained and compared with scans taken under “no task” conditions, during which the animals watched a video. On the day of PET imaging, NHPs were placed in primate chairs, and the DMS task was initiated. After 10 trials, NHPs received a 30-s injection of 3–5 mCi (0.111–0.185 MBq) of [^{18}F]-2-deoxy-2-fluoro-D-glucose (FDG) and then performed the task for another 40 min, after which they were anesthetized with ketamine (15 mg/kg i.v.) and placed in the PET scanner. After PET imaging, the NHPs were returned to their home cages. DMS testing was resumed the following day. Three blood samples were collected from the femoral vein opposite the FDG injection site 10 min prior to FDG injection and at 8 and 42 min postinjection. Tracer concentrations were measured using an automated gamma well counter. The CMR_{glc} in each region of interest was calculated using a population-averaged curve as described previously (15–19).

Scans were acquired on a General Electric (GE Medical Systems, Milwaukee, WI) Advance NXi PET scanner with 4 mm resolution. A 5-min 2D transmission scan was acquired for attenuation correction, followed by a 10-min 3D emission scan. Transmission scan data were smoothed (4-mm Gaussian filter) and the attenuation-corrected emission data were reconstructed into 128×128 matrices (Hanning filter with a 4-mm cutoff transaxially and a ramp filter with an 8.5-mm cutoff axially).

PET data were analyzed with Statistical Parametric Mapping (SPM99) software implemented in MATLAB (MathWorks, Natick, MA) as described previously (15). Briefly, reconstructed images were coregistered with the corresponding structural MR images obtained previously on a 1.5 T MR scanner (GE Medical Systems) (15) using automated image registration (20) and then transformed spatially into a standard space with a rhesus monkey FDG template constructed in our laboratory (15). Effects at each voxel were estimated according to the general linear model using the multisubject and covariate options in SPM. Statistical maps were derived for comparisons of the scans prior to and after fractionated whole-brain irradiation. Areas of activation were displayed on a T₁ weighted anatomic MRI template, and the regions were identified with reference to standard atlases of the NHP brain as described previously (20). Statistical significance was determined using a *P* value of <0.001, corrected for search volume.

RESULTS

The DMS performance of the three NHPs did not vary over the year prior to fractionated whole-brain irradiation and was similar to that of other age-matched untreated NHPs used in separate projects over recent years (21). Only the last 4 months are shown in Fig. 2. All three NHPs showed the same pattern of changes in performance on the DMS task after irradiation, so the data were combined for analysis. Figure 2 shows the change in overall performance (mean percentage correct $\pm$ SEM) for trials with increasing cognitive load from two to six images summed across the three NHPs after irradiation relative to their levels before irradiation ($F_{(1, 2577)} > 9.14$, $P < 0.001$, ANOVA_2d). DMS trials performed using only two images (low cognitive load) did not show a statistically significant reduction in low load cognitive performance relative to the levels before irradiation until 7 months after irradiation. After this time, performance levels declined progressively over the remaining testing period (Fig. 2). In high cognitive load trials, the initial impairment was observed ~1 month after irradiation. This was followed by a transient recovery period over the next 1–2 months, after which performance declined progressively through 11 months after irradiation. The severity of this progressive decline in cognitive performance was a direct function of the cognitive load of a given trial as indicated by the difference in the rate of decline in the mean percentage of correct trials for two vs six images averaged over the 11-month testing period (Fig. 2, inset).

Analysis of the three NHP FDG-PET scans performed prior to fractionated whole-brain irradiation (Fig. 3) showed that the brain regions exhibiting increased CMR_{glc} [dorsal prefrontal cortex (DPFC), medial temporal lobe (MTL), parietal cortex (precuneus), thalamus, and dorsal striatum (DStr))] during the DMS task compared to those during the non-task were essentially identical to those reported previously (15, 21). These brain regions have repeatedly been implicated in human and NHP cognitive function (22, 23). When the FDG-PET analysis during the DMS task was repeated 9 months after irradiation, the mean CMR_{glc} in the cuneate cortex and DPFC regions was less than that prior to irradiation, indicating less glucose metabolism in these critical brain regions that were previously engaged in the DMS task (Fig. 4, top row). In contrast, the mean CMR_{glc} increased in the cerebellum and thalamus, regions that were not engaged in the DMS task prior to irradiation (Fig. 4, bottom row).

DISCUSSION

Studies with rodent models have established that fractionated whole-brain irradiation leads to a significant and progressive decline in both hippocampal- (24) and non-hippocampal- (25) dependent cognitive function 6–12 months after fractionated whole-brain irradiation that can be prevented or ameliorated by drugs prescribed for type II diabetes (26) and

hypertension (25). However, differences in the structure and organization of the rodent and human brain (8) as well as the difficulty of measuring higher-order executive functions in rodents make it difficult to translate these findings to the clinic. In contrast, NHP models allow examination of higher-order cognitive processes and behavioral tasks similar to those of humans (11). The DMS task used in this study represents an important tool with which to serially assess the impact of fractionated whole-brain irradiation on higher-order cognitive functions that require participation of the same brain regions (15, 21) implicated in human cognition (22, 23).

The current data demonstrate that adult male NHPs exposed to a fractionated whole-brain radiation dose that is expected to be biologically similar to the dose delivered to glioma patients exhibit a significant reduction in higher-order cognitive function. The time course and severity of this reduction in performance depend on the cognitive load. In general, the decrease in cognitive performance on low-load trials becomes statistically significant later than the decrease on high-load trials (Fig. 2). Thus these chronic radiation-induced decreases in higher-order cognitive function after fractionated whole-brain irradiation appear to be similar to the decreases in memory and executive function measured in patients 6 months or more after fractionated whole-brain irradiation (4).

The effect of fractionated whole-brain irradiation on brain function was also investigated by determining the glucose uptake in various brain regions during the DMS task. The CMR_{glc} measured with FDG-PET in a brain region is an indicator of the level of neurosynaptic activity in that region. This has become a standard technique in both oncology and dementia research (27) and has been employed previously with NHPs to determine alterations in DMS task-related brain activity after sleep deprivation and its modulation by ampakines (21). Those studies revealed that the brain regions activated during the DMS task were the DPFC, MTL and DStr, areas repeatedly implicated in cognitive function in humans and NHPs (22, 23). When cognitive function was impaired at 9 months after fractionated whole-brain irradiation, glucose metabolism in the cuneate and DPFC regions was reduced compared to the glucose metabolism before irradiation (Fig. 4). In contrast, glucose metabolism in the cerebellum and thalamus, regions that were not engaged during the DMS task before irradiation, was increased (Fig. 4), indicating a potential imbalance in frontal-subcortical circuits (28). This decreased capacity to perform higher-order executive functions has been shown to involve the DPFC, hippocampus, cuneate and motor areas in cancer survivors after fractionated whole-brain irradiation (3).

We recognize that these initial findings require verification. Further studies using larger numbers of animals as well as sham-irradiated age-matched controls are in progress to confirm and extend these initial observations. However, the results of this pilot study do indicate that the changes in higher-order cognitive function and brain metabolism induced by fractionated whole-brain irradiation observed in the rhesus monkey appear to be similar to those observed in brain tumor patients after radiotherapy. Given that NHPs also have ~95% overall genetic coding sequence identity to humans (29), have anatomical and physiological systems that are similar to humans in structure and function, and are excellent models of various human diseases and conditions that increase in frequency with age (30), they represent an important translational model for studying radiation-induced cognitive impairment and interventions to modulate it.

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FIG. 1.

The delayed-Match-to-Sample task: (1) display for initiation of the trial (circle), (2) presentation of Sample image, (3) blank screen during a 1–30-s delay, and (4) presentation of Sample image plus two distracter images. All images are clip art and are unique for each trial within a session. Trials with longer delays and more distracter images have a higher cognitive load than trial with short delays and fewer distracter images.

FIG. 2.

Fractionated whole-brain irradiation leads to chronic, progressive cognitive impairment. Each bar represents the mean percentage ($\pm$ SEM) of correct trials with two to six images summed over all animals, trials and daily sessions for each month. Arrows indicate the start of irradiation. ** $P < 0.001$; horizontal bars span months where asterisks apply. The inset shows a regression analysis of the average monthly performance of the three NHPs at low (two images) and high (six images) cognitive load.

FIG. 3.

Analysis of the preirradiation [^{18}F]FDG-PET scans of cerebral glucose metabolic activity during the DMS task compared to that during the non-task plotted on T_1 MR images. The areas in red represent the average increase in glucose metabolism during the DMS task. The color bar is the degree of intensity difference, shown as a scale of t values with $P < 0.001$.

FIG. 4.

[^{18}F]FDG-PET scans of cerebral glucose metabolism 9 months after fractionated whole-brain irradiation. Upper panel: Postirradiation < Preirradiation. Blue areas in the cuneate cortex and prefrontal cortex exhibited less metabolic activity in the scans obtained 9 months after irradiation than in the scans prior to irradiation. Lower panel: Postirradiation > Preirradiation: The red areas in the cerebellum and thalamus showed greater metabolic activity in the scans obtained 9 months after irradiation than in those obtained prior to irradiation. The color bar is the degree of intensity difference shown as a scale of t values with $P < 0.001$.