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DISCLAIMER

RADIATION-INDUCED PERTURBATIONS IN BLOOD-BRAIN BARRIER AND REGIONAL CEREBRAL BLOOD FLOW IN THE RABBIT BRAIN[1]

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The rabbit brain was used as an animal model to examine the underlying pathophysiologic mechanisms of delayed radiation injury in the brain. At 2.5 mo following high-dose irradiation with 60 Gy (225 keV X-rays) to the left hemisphere, *in vivo* GdDTPA-enhanced nuclear magnetic resonance (NMR) scans demonstrated focal regions of contrast enhancement in the corona radiata, hippocampus, and fimbria, indicating the presence of blood-brain barrier (BBB) disruptions. Regional cerebral blood flow (rCBF) patterns were measured with radioactive microspheres; the right hemisphere was used as an internal control. Left-right (irradiated-unirradiated) rCBF ratios were less than 1.0, demonstrating decreased rCBF in the irradiated hemisphere. Although it has been previously suggested that delayed radiation injury in the high-dose regimes is primarily mediated by injury to the oligodendroglial cell population, these results demonstrated that perturbations in cerebrovascular function may also occur, and these perturbations may represent secondary reactions to metabolic cell injury in the irradiated brain.

Keywords: delayed radiation injury, blood-brain barrier, regional cerebral blood flow, nuclear magnetic resonance, microspheres, X-rays, brain

The delayed reaction to radiation injury in the brain presents a serious potential complication in the radiosurgical and radiotherapeutic approach to intracranial disorders. The pathophysiologic mechanisms underlying this complex phenomenon are not well-understood, but have been demonstrated to involve a wide spectrum of cerebrovascular, metabolic, and functional perturbations[4,8,9,14,18]. Prior studies have demonstrated that the irradiated rabbit brain serves as a suitable animal model for investigating this difficult problem[5,13,14,19]. Following hemibrain irradiation with 30 Gy helium-ions, the timing and extent of focal lesions in the irradiated hemisphere could be observed with *in vivo* nuclear magnetic resonance (NMR) and positron

emission tomography (PET) scanning techniques[13,14]. In this report, perturbations in cerebrovascular function in the rabbit brain were examined following high-dose irradiation. Initial results demonstrated that early radiation-induced alterations in regional cerebral blood flow (rCBF) occurred in irradiated brain, and these alterations correlated well with blood-brain barrier (BBB) disruptions observed with *in vivo* NMR imaging scans.

A total of 9 New Zealand White rabbits (6-9 mo old) were studied: 4 unirradiated controls; and 5 irradiated animals. Rabbits were irradiated with a 225 keV X-ray machine (Philips Medical Systems). A Victoreen ionization chamber was used for radiation dosimetry and calibrations. All animals were anesthesized with 32 mg/kg Ketamine, 3 mg/kg Rompun, 0.6 mg/kg Acepromazine for the irradiation procedures. Rabbits were placed in a prone position, and the X-ray beam port was positioned directly over the dorsal surface of the left side of the head. A 20 mm x 10 mm hemicircular port was used to restrict radiation to the left hemisphere. A skin surface dose of 75 Gy was used, resulting in an average brain dose of about 60 Gy at 1 cm depth. Dose to the ventral base of the brain (approximately 3 cm depth) was about 30 Gy. The dose rate was approximately 1.8 Gy/min.

NMR imaging studies were performed on a GE 1.5 Tesla Signa Imaging System, using techniques previously described[13,14]. Briefly, rabbits were anesthesized with the same Ketamin/Rompun/Acepromazine mixture, and placed in a prone position in the NMR imaging magnet. Approximately 1-1.5 mmol/kg of gadolinium diethylaminetriaminepetnaacetic acid (GdDTPA) was injected into a marginal ear vein for each study. T1-weighted (T1W) spin-echo scans were used (TR 400 ms, TE 25 ms) so that regions of BBB disruption would appear as areas of contrast enhancement and hyperintense signal. Rabbits were scanned at 1 dy post-irradiation, 1 wk post-irradiation, and subsequently once every 2 wks.

Styrene-divinyl resin microspheres (NEN-TRAC Dupont), 15 μm in diameter

and labelled with ^{57}Co, were used for rCBF measurements. The microspheres have a relative density of approximately 1.4, similar to that of blood. When injected into the bloodstream, the microspheres have similar flow characteristics to that of red blood cells, and are randomly distributed over arterial cross-sections. The radioactive label enabled quantification of microspheres trapped in brain tissue, thus allowing for calculation of rCBF values. The microspheres were suspended in saline with 1% Tween as a surfactant. All microsphere suspensions were sonicated in an ultrasonic cleaner to prevent clumping and ensure a uniform suspension prior to use. Aliquots of 350,000 to 450,000 microspheres (approximately 6-8 μCi) were used for each study, resulting in about 400-500 spheres per brain tissue sample. This allowed for approximately 95% confidence limits within a 10% error interval of calculated rCBF values[1,7].

Four control rabbits were used for measuring baseline rCBF values and variances in normal rabbit brain. Two irradiated rabbits were examined at 1 mo post-irradiation, and three irradiated rabbits were examined at 2.5 mo post-irradiation. Rabbits were anesthesized with the Ketamine/Rompun/Acepromazine mixture, and the chest and right hind leg regions were shaved and cleaned with alcohol. A 2-inch 22-gauge catheter was inserted into the femoral artery to establish an arterial line. Reference blood withdrawal, at 3.2 ml/min from the femoral artery, was begun 10 s before the microspheres were injected. Microspheres were injected as a bolus into the left heart ventricle using a 1.5-inch 18-gauge syringe needle inserted between the ribs through the chest wall. Proper placement of the syringe needle in the left ventricle was confirmed with a Statham P23D pressure transducer. Reference blood withdrawal was continued at least 40 s after the microsphere injection was completed. The rabbits were killed with a lethal overdose of sodium pentobarbitol (Nembutol), and the brains were excised immediately.

The rabbit brain was dissected into left and right frontal lobe, parietal lobe,

temporal lobe, occipital lobe, hippocampus and fimbria, thalamus, striatum, and cerebellar hemispheres. It was not possible to dissect out consistently the fimbria from the hippocampus, so they were counted as a single region. Also, no attempt was made to seperate out the white matter tracts of the corona radiata from the gray matter of the cerebral cortex.

Tissue samples and the reference blood sample were weighed and placed into plastic counting tubes. Left and right kidney samples were also obtained to ensure proper left-right distribution of microsphere flow. Left and right lung samples were obtained to ensure proper trapping of the microspheres in the first pass through the brain without abnormal arteriovenous shunting (i.e., counts in lung samples should be low). All samples were counted in a well-type gamma counter adjusted for ^{57}Co decay photons. Quantitative values of rCBF were calculated using standard techniques[11] where

$$rCBF = \frac{sample\ counts}{reference\ blood\ counts} \times \frac{reference\ blood\ withdrawal\ rate}{sample\ weight} \times 100$$

NMR imaging studies of all 4 control rabbits demonstrated no brain abnormalities. Rabbits irradiated with 60 Gy (225 keV X-rays) demonstrated no changes on GdDTPA-enhanced NMR scans up through 2 mo post-irradiation. Except for one rabbit which demonstrated a slight head tilt to the left (irradiated) side after about 1 mo, none of the rabbits showed any neurologic deficits. At approximately 2.5 mo post-irradiation, well-defined areas of contrast enhancement in the irradiated hemisphere were observed on GdDTPA-enhanced NMR scans, indicating the presence of focal disruptions of BBB integrity (Figure 1). These perturbations were located primarily in the white matter regions of the corona radiata and some parts of the adjacent cortical gray matter. The hippocampus and fimbria also appeared to be involved, but other deep brain structures and nuclei were not affected.

There was no macroscopic evidence of tissue necrosis visible upon dissection

of the irradiated brains for microsphere rCBF studies. Microsphere flow measurements of rCBF in control rabbits demonstrated a range of values of about 140-190 ml/100g/min in the cortical regions, 80-90 ml/100g/min in the hippocampus, 110-150 ml/100g/min in the thalamus and striatum, and 90-110 ml/100g/min in the cerebellar hemispheres (Table I). The highest rCBF values were found in the occipital lobe; this was most probably an effect of the Ketamine anesthesia. Ketamine-induced changes in cerebral metabolism and blood flow have been found to occur in the occipital cortex, superior colliculus, and limbic brain regions[3,6]. The absolute rCBF values were slightly higher than values previously obtained in other studies[12,15]. This may have been due to the fact that reference blood withdrawal was conducted through the femoral artery, resulting in slightly lower microsphere extraction rates than previous studies where reference blood withdrawal was performed from the descending aorta. As a result, left-right (irradiated-unirradiated) rCBF ratios were used; this method of data analysis also had the advantage of normalizing for systemic variations. Left-right rCBF ratios were approximately 0.9 to 1.1 in all structures, with a slight tendency for rCBF values to be somewhat higher in the left hemisphere for cortical brain regions.

Absolute rCBF values at 1 mo post-irradiation did not demonstrate significant differences from the control animals (Table I). However, at 2.5 mo post-irradiation, there appeared to be a decrease in rCBF values in the irradiated hemispheres (Table I), although absolute rCBF values in the irradiated brains demonstrated large standard deviations, reflecting both the heterogenous and complex response to radiation injury, as well as the small population studied in these initial investigations. Perturbations in rCBF were especially prominent in the cortical regions. Lesser decreases in rCBF were observed in the thalamus and hippocampus. No significant differences in rCBF were demonstrated in the striatum and cerebellum.

Significant differences ($p<0.05$, Mann-Whitney test) in left-right rCBF ratios between irradiated brains and unirradiated controls were demonstrated in the frontal, parietal, and occipital cortical brain regions (Figure 2). Although 2 of the 3 rabbits measured at 2.5 mo post-irradiation demonstrated significantly decreased flow in the irradiated temporal lobe (average left-right rCBF ratio of 0.78), the large standard error for this brain region (Figure 2) was due to the fact that 1 rabbit had a left-right rCBF ratio of 1.21. Since the temporal lobes lie on the lateral and distal edges of the radiation field, it is possible that slight errors in positioning may have resulted in a lower dose to this brain region in this 1 rabbit.

It has been proposed that cerebrovascular injury may mediate the processes of delayed radiation injury in the brain at lower doses (20-30 Gy) whereas, at high doses (greater than 30-40 Gy), damage to the oligodendroglial cell population predominates, and the development of parenchymal necrosis occurs before the induction of cerebrovascular injury[18]. It has been demonstrated in the rat brain that, following irradiation with 20-25 Gy X-rays, histopathological alterations in the cerebrovasculature appear 3-4 mo prior to parenchymal necrosis[2]. Studies with I^{131}-iodoantipyrine extraction techniques have suggested that alterations in rCBF occur as early as 3 mo after irradiation of the rat brain with 20-30 Gy X-rays[16]. At higher doses (greater than 40 Gy), no changes in iodoantipyrine extraction occurred up through a year post-irradiation[16]. Studies using free radical scavengers as radioprotective agents demonstrated that these agents, which do not cross the BBB and therefore only affect cerebral endothelial cells, may protect against radiation necrosis only at low-dose regimes[17].

In this investigation, marked alterations in rCBF and BBB integrity were observed in the rabbit brain 2.5 mo following high-dose irradiation with 60 Gy X-rays. These early perturbations in cerebrovascular function were primarily localized to the cortex, corona radiata, hippocampus, and fimbria, and did not involve deep brain

structures and nuclei. To a lesser degree, rCBF was also decreased in the thalamus, although no BBB disruptions in this brain region were noted on *in vivo* NMR scans. These rCBF alterations in the thalamus may have been due to metabolic diaschisis or other transnueronal mechanisms related to the cerebrovascular perturbations in the cortex and hippocampus.

Although consistent decreases in rCBF were observed in the irradiated rabbit brain, these decreases of only 20-40% should not induce direct parenchymal infarction and injury; it has been shown that decreases in rCBF of up to 50-60% may be tolerated without functional impairment or cellular necrosis[10]. It appears that these rCBF perturbations represent early changes in cerebrovascular function, and may be secondary reactions to metabolic perturbations in myelin maintenance and renewal processes in the oligodendroglia. Thus, these results do not contradict earlier studies, but rather, demonstrate that the cerebrovascular system is quite sensitive to injury, even at higher doses, and may undergo early pathologic alterations in function that precede but do not directly induce the appearance of demyelination and parenchymal necrosis. These secondary changes in cerebrovascular function and integrity would account for the decreased rCBF in the irradiated hemisphere, and the contrast enhancement observed on GdDTPA-enhanced NMR imaging procedures. Widespread parenchymal injury and necrosis may develop later with a longer latent period.

One limitation of the microsphere flow method was that it was unable to measure flow differences in gray matter and white matter regions of the cortex; it was not possible to consistently dissect out white matter tracts because the rabbit brain has a very small white matter component. Ongoing experiments are being performed to correlate microsphere flow measurements with hydrogen clearance rCBF techniques in order to measure blood flow alterations in the white matter brain regions. Also, histologic studies of these radiation-induced changes are be-

ing conducted for correlation with the rCBF measurements and the NMR imaging results.

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Figure Legends

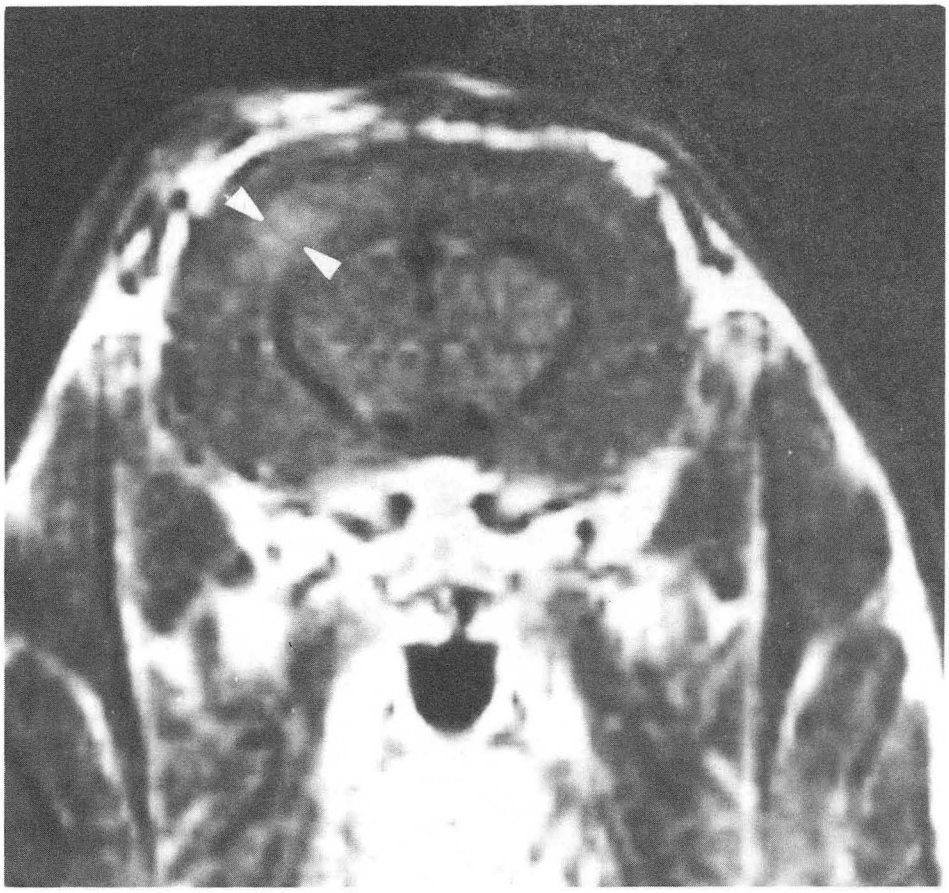
Figure 1. Coronal GdDTPA-enhanced NMR scan of rabbit brain at 2.5 mo following left hemibrain irradiation (60 Gy, 225 keV X-rays). A focal region of contrast enhancement (arrowheads) was observed in the irradiated left hemisphere, indicating a region of BBB disruption. The lesion was limited primarily to the corona radiata, hippocampus, and fimbria, although some enhancement of the adjacent cortical gray matter was also observed. There were no abnormalities in the deeper brain structures and nuclei (striatum, thalamus, and hypothalamus).

Figure 2. Radiation-induced alterations in left-right (irradiated-unirradiated) rCBF ratios (mean+S.E.M.) in the rabbit brain. At 2.5 mo following hemibrain high-dose X-irradiation (60 Gy), blood flow to the irradiated left hemisphere was decreased, especially in the cortical lobes, as demonstrated by left-right rCBF ratios which were less than 1.0 (striped bars). Unirradiated controls (unstriped bars) demonstrated left-right rCBF ratios which were greater than 1.0, indicating that blood flow to the left cortex was actually greater than blood flow to the right cortex in normal brain. The large standard error for the irradiated temporal lobe was because 2 irradiated brains demonstrated left-right rCBF ratios of 0.78, but 1 irradiated brain had a left-right rCBF ratio of 1.21. (FR = frontal lobe; PA = parietal lobe; TE = temporal lobe; OC= occipital lobe).

TABLE 1

REGIONAL CEREBRAL BLOOD FLOW (ml/100g/min) IN CONTROL AND IRRADIATED BRAIN (Mean±S.D.)

	CONTROL BRAIN		IRRADIATED BRAIN (1 mo)		IRRADIATED BRAIN (2.5 mo)	
	Left Hemisphere	Right Hemisphere	Left Hemisphere	Right Hemisphere	Left Hemisphere	Right Hemisphere
Frontal Lobe	158.0±1.0	146.9±10.9	170.8±31.8	144.2±37.8	119.4±28.8	167.6±45.3
Parietal Lobe	153.9±7.4	150.0±15.2	119.6±19.7	117.7±44.2	117.4±17.3	186.6±35.4
Temporal Lobe	164.7±9.9	145.9±11.7	127.5±21.5	138.7±11.6	122.0±35.5	124.1±11.2
Occipital Lobe	197.7±9.4	194.1±14.1	194.6±17.8	182.5±18.1	127.7±12.7	196.1±18.2
Hippocampus	88.6±1.5	85.2±2.1	87.8±8.1	74.0±2.9	72.3±2.2	86.6±7.6
Thalamus	113.1±15.7	112.5±13.9	90.33±14.9	72.7±15.5	95.8±19.9	118.8±25.5
Striatum	144.9±21.0	148.5±9.0	118.6±6.8	91.5±27.2	121.8±32.6	128.4±31.9
Cerebellum	114.7±7.2	100.0±3.1	107.7±6.8	104.9±6.6	102.2±4.1	103.7±1.6



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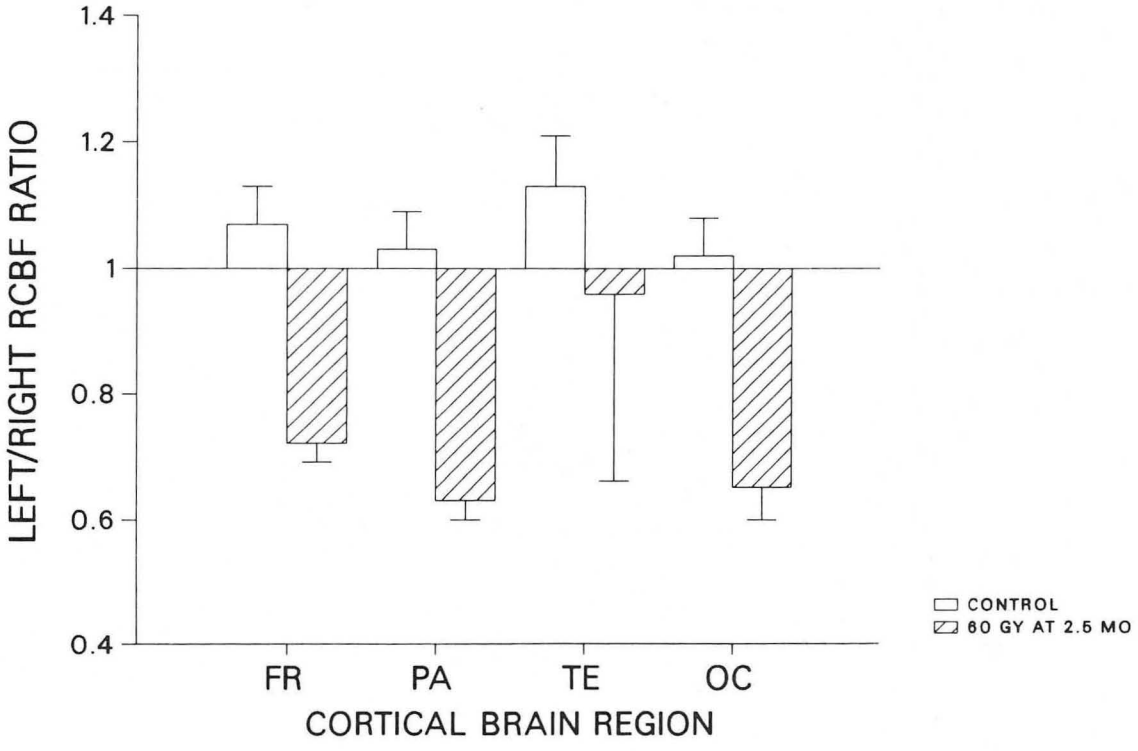

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