LIPID PEROXIDATION DOES NOT APPEAR TO BE A FACTOR IN LATE RADIATION INJURY OF THE CERVICAL SPINAL CORD OF RATS

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Purpose: We tested the role of lipid peroxidation in the demyelination and white matter necrosis associated with radiation injury of the central nervous system.

Methods and Materials: We irradiated the cervical spinal cords of female F344 rats (23 Gy) and assayed for the accumulation of the peroxidation byproducts malondialdehyde and hydroxyeicosatetraenoic acids, and for the consumption of the endogenous free radical scavengers vitamins E and C. We further tested the role of lipid peroxidation in radiation injury of the central nervous system by determining the sensitivity of the cervical spinal cord to radiation in rats on diets containing deficient, normal, and supplemental levels of the antioxidant vitamin E. Rats were placed on these diets at 4 weeks of age and irradiated (18.5–21.5 Gy) 16 weeks later.

Results: During the 5 months between irradiation and the onset of paralysis, no accumulation of peroxidation byproducts or consumption of endogenous scavengers was seen in the cervical spinal cords of the irradiated rats. The cervical spinal cords of some of the rats placed on the diets with deficient, normal, and supplemental levels of vitamin E were analyzed at the time of irradiation and contained 197 ± 57, 501 ± 19, and 717 ± 35 pmol vitamin E/mg protein, respectively. Despite the statistical differences in these levels, the radiation sensitivity of the cervical spinal cord (ED50, for white matter necrosis) in rats receiving the three diets was not different (20.4, 20.7, and 20.6 Gy).

Conclusion: These data do not support a role for free radical-induced lipid peroxidation in the white matter damage seen in radiation injury of the central nervous system.

Central nervous system, Radiation injury, Tocopherol, Lipid peroxidation, Rat spinal cord.

INTRODUCTION

Interstitial brachytherapy is effective both in the treatment of selected patients with recurrent malignant gliomas (16, 28) and as a “boost” after external irradiation for glioblastoma multiforme (17). However, in approximately half of these patients treated with brachytherapy, surgical resection is later required for focal radiation necrosis (16, 17, 28). Successive follow-up computerized tomography (CT) scans show evidence of increasing contrast enhancement (injured white matter) corresponding to the site of implantation in these patients (16, 28) (Fig. 1). A spreading wave of decreased density emanates from the necrotic zone and progresses through the white matter of regions far removed from the implant site (Fig. 1), sometimes causing profound neurological deterioration.

The brains of patients treated with brachytherapy have undergone detailed neuropathologic study at autopsy by our group (8). Demyelination is clearly the underlying pathological correlate of the radiological changes and for the progressive clinical deterioration seen in these patients. Late radiation injury of the brain caused by irradiation from any source is known to have a marked propensity for the white matter and is typically expressed as demyelination (6, 8, 9, 39). The pathogenesis of this demyelination has been a topic of debate for decades, possible target...
Fig. 1. Computerized tomography scans of a recurrent right parietal glioblastoma before (left), 1 month after (middle), and 1 year after (right) interstitial brachytherapy. Progressively increasing contrast enhancement and perilesional edema secondary to radiation necrosis is apparent. Radiation necrosis was seen at reoperation.

cells are the endothelial cells, the oligodendroglia, or both (22, 39). Although a loss of glial cells to a critical threshold may account for the white matter necrosis seen in radiation injury, this explanation alone has been questioned as too simple (35). Autoimmune mechanisms may also play a part in the destruction of myelin.

In comparison, there has been little interest in the biochemical mechanisms that may cause or contribute to late brain injury. Estable-Puig and coworkers (13) showed a progressive reduction in the myelin "stainability" (de-myelination) of the cerebral white matter of rats, which was well advanced within 72 hr after exposure to high doses of α particles. Target cell injury was considered unlikely to account for the changes seen so early after injury; instead, a direct effect of radiation on the lipid-rich myelin, such as the formation of lipid hydroperoxides and a subsequent chain reaction of lipid peroxidation, was postulated (13). Such a chain reaction has been shown through in vitro experiments in model membranes, in which irradiation produces oxygen derived radicals (most importantly HO·) that initiate peroxidation reactions in polyunsaturated fatty acids such as arachidonate (1, 32). The secondary generation of less reactive, but still potent, lipid peroxide radicals then propagates the peroxidation. The intensity of these peroxidation reactions is inversely proportional to the dose rate of irradiation (32).

In central nervous system (CNS) tissue, radiation exerts its effect in part through reactive molecules derived from molecular oxygen that can peroxidize polyunsaturated lipids in cell membranes (1, 2, 4, 31, 32). Cells of the CNS have a high degree of unsaturation of lipids (5), and oxygen radicals and lipid peroxidation have been implicated in traumatic (19), ischemic (42, 43), and even degenerative (10, 20) CNS diseases. Hornsey et al. (23) treated rats with irradiated cervical spinal cords with desferrioxamine and a low iron diet and showed that this regimen was radioprotective. These investigators felt that the protection was against a radiation-induced, post-ischemic reperfusion injury caused by oxygen-derived free radicals, at least part of the injury being caused by peroxidation of lipids. Lipid peroxidation has not been measured in the irradiated CNS, and it is unknown how long lipid peroxidation reactions can persist in CNS tissue. The longest previous observations of such reactions spanned only 5 days in the spinal cords of cats after impact trauma (36).

Therefore, to determine whether the progressive radiation injury in lipid-rich cerebral white matter results from a similar peroxidation chain reaction, we investigated the role of lipid peroxidation chain reactions in late delayed radiation injury of the CNS. We hypothesized that the progressive, spreading destruction of lipid-rich white matter seen after brachytherapy in some patients was caused by a wave of ongoing lipid peroxidation. The inverse relationship between the intensity of peroxidation and dose rate (32) gives credence to this theory, as brachytherapy is a low dose rate application. Moreover, it seemed that ongoing chain reactions of lipid peroxidation might also help to explain the long latent interval associated with radiation injury to the CNS caused by conventional teletherapy.

Because the paralysis attending spinal cord radiation injury in the rat is abrupt and predictable relative to the vague external signs of brain radiation injury (9, 39), we investigated peroxidation in the rat spinal cord rather than brain. To detect lipid peroxidation in the irradiated cervical spinal cords of rats during the months between a dose of radiation and the resultant paralysis, we assayed for accumulated byproducts of lipid peroxidation (malondialdehyde [MDA] and hydroxyeicosatetraenoic acids [HETEs]) and for consumption of endogenous cellular antioxidants (vitamins E and C). To test further whether lipid peroxidation was involved in radiation injury to the CNS, we measured the response to radiation in the cervical spinal cord of rats on long-term diets supple-
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mented with vitamin E, normal in vitamin E, and deficient in vitamin E.

METHODS AND MATERIALS

Animal irradiation

Female F344 rats, 70–80 days old, were anesthetized with nembutal (30 mg/kg i.p.), and their cervico-thoracic spinal cords were irradiated using a dorsal port of 5 × 15 mm at the C2-T1 spinal levels. Irradiation was done in single fractions using 250 kVp x-rays (half value layer 1.8 mm Cu) at a dose rate of 4.24 Gy/min. Twelve to eighteen animals were used for each dose point. The dosimetry was confirmed with a plastic phantom incorporating thermoluminescent dosimeters.

After treatment the animals were weighed weekly and watched closely for a minimum of 7 months until forelimb or hindlimb paralysis developed. Dose-response curves were constructed by logit analysis with correction for censored data (41), and the ED50 for white matter necrosis (forelimb or hindlimb paralysis) was determined. In addition, the irradiated segment of the spinal cords from some of the paralyzed animals was analyzed histopathologically.

Biochemical assays

Assays were performed for the accumulation of by-products of the peroxidation pathway and for the consumption of endogenous antioxidants. Rats were irradiated with single doses of 23 Gy. Rats were sacrificed at various intervals after irradiation, the spinal cords were removed, and the irradiated segments were analyzed biochemically. Unirradiated age-matched rats served as controls.

MDA levels were determined using a standard thio-barbituric acid assay (37). Phospholipids and free fatty acids were extracted from the spinal cords, and the HETEs were assayed using reversed phase high-performance liquid chromatography (RP-HPLC) as described by Cashman et al. (7) and Yamamoto et al. (43, 44). High-performance liquid chromatography with in-line ultraviolet and electrochemical detection was used to determine the level of vitamin E (27). The level of vitamin C was assayed using a paired-ion, RP-HPLC procedure with electrochemical detection and internal standard quantitation based on isoascorbic acid (26).

Vitamin E dietary manipulation

Groups of rats of 4 weeks of age were fed chow* supplemented with vitamin E (diet A, 1000 ppm vitamin E), normal in vitamin E (diet B, 50 ppm vitamin E), or deficient in vitamin E (diet C, <1 ppm vitamin E) for 16 weeks before irradiation, and the ED50 for white matter necrosis was determined from dose-response curves as described above. The rats were kept on their respective diets after irradiation until paralysis, except for the group receiving diet C, in which diet B was given for 4 days at 8 weeks after irradiation because of the animals’ failure to thrive (weight loss). They were then given a diet containing 7.5 ppm vitamin E until paralysis.

Some animals on each diet were killed at the time of irradiation and at the time of paralysis, and their cervical spinal cords were removed for analysis of vitamin E content.

RESULTS

Histopathology

Histopathological analysis of spinal cords taken from paralyzed animals confirmed the presence of demyelination and necrosis throughout the white matter in the irradiated segment (Fig. 2). These processes showed a particular predilection for the posterior columns.

Assays for lipid peroxidation

During the 5 months after irradiation, the levels of MDA and HETEs in the irradiated cord segments were not different from the levels in unirradiated cords (Figs. 3 and 4), except for a lower level of MDA at 3 months in the irradiated group (p = 0.044). Similarly, the levels of vitamins E (Fig. 5) and C (Fig. 6) in the spinal cords of irradiated animals were not statistically different from the levels in control animals except for a lower level of vitamin E at 1 month after irradiation (p = 0.02) (Fig. 5) and a lower level of vitamin C at 4 months after irradiation (p = 0.009) (Fig. 6).

Dietary manipulation of vitamin E intake

The vitamin E levels in the spinal cord at the time of irradiation after 4 months on the respective diets were

* Dyets, Inc., Bethlehem, PA.
Fig. 3. Levels of MDA in irradiated and unirradiated (control) rat cervical spinal cords at intervals between irradiation and paralysis. The MDA level was significantly lower in the irradiated spinal cords at 3 months (*) \((p = 0.009)\). Error bars represent standard error of the mean.

717 ± 35 pmol/mg protein for rats receiving diet A, 501 ± 19 pmol/mg protein for those on diet B, and 197 ± 57 pmol/mg protein for those on diet C. The levels are significantly different (diet A vs. diet B, \(p < 0.05\); diet B vs. diet C, \(p < 0.05\)). At the time of paralysis, the spinal cord vitamin E levels were higher overall and remained highest in rats on diet A and lowest in rats on diet C (diet A vs. diet B, \(p < 0.05\); diet B vs. diet C, \(p < 0.05\)).

The dose response curves for white matter necrosis in irradiated animals on the various diets virtually overlapped (Fig. 7). The ED\(_{50}\) values were nearly identical at 20.6 Gy (95% confidence, 20.2–21.1 Gy) for diet A, 20.4 Gy (95% confidence, 20.1–20.6 Gy) for diet B, and 20.7 Gy (95% confidence, 20.4–21.1 Gy) for diet C.

**DISCUSSION**

We investigated the possibility of ongoing peroxidation in irradiated cords on several levels, the first of which was by assaying for peroxidation products. Elevated levels of MDA, perhaps the most studied byproduct of free radical peroxidation of unsaturated fatty acids (36), have been used to support a role for free radical reactions in several models of CNS injury (36,43). However, we documented no increases in MDA levels in the spinal cords of our irradiated animals compared with the levels in control animals (Fig. 3). At 3 months, the level of MDA was actually lower in the irradiated cords. Elevation of HETEs, a more specific indicator of membrane lipid peroxidation than elevation of MDA (7, 44), was not seen either (Fig. 4). Moreover, no evidence of consumption of the endogenous free radical scavengers vitamin E (11) and vitamin C (11, 21) between radiation and paralysis was seen (Figs. 5 and 6). Although sporadic differences from control levels were seen in the vitamin E and vitamin C levels, as in the MDA measurements, these changes were not consistent. We attribute these differences to biological variability and...
That antioxidants can be radioprotective. Vitamin E injury by acting as radioprotectors. Vitamin E, because of its high lipid solubility, would seem to be the ideal antioxidant to test as a CNS radioprotector (40). The ability of vitamin E to act as an antioxidant in vivo, protecting tissue lipids from attack by free radicals, is its key biological function and one of the cell's major defenses against free radicals (29, 38). Vitamin E is absorbed from the gut, and it is distributed to all tissues, the ultimate levels depending on the amount given and the duration of administration (30). Because of the importance of the duration of administration, the dietary route is the most effective method for raising levels of vitamin E in the CNS (3).

Fig. 7. Dose response for paralysis for rats fed for 16 weeks on diets supplemented (diet A, 1000 ppm vitamin E; solid line), normal (diet B, 50 ppm vitamin E; short-dashed line), and deficient (diet C, <1 ppm vitamin E; long-dashed line) in vitamin E and then irradiated to their cervical spinal cords. Error bars denote 95% confidence limits about the ED50.

Evidence from both in vitro and in vivo systems shows that antioxidants can be radioprotective. Vitamin E inhibits peroxidation in irradiated model membrane systems of liposomes (24), and supplemental vitamin E amplifies radioprotection by formate, mannitol, some alcohols, and other compounds in another in vitro membrane system (33). In vivo radioprotection by antioxidants is supported by increased sensitivity to irradiation (decreased LD50) and greater vulnerability to lipid peroxidation in vitamin E-deficient mice than in normal mice (25). Injection of glutathione and cysteamine before irradiation reduces vulnerability to irradiation in these vitamin E-deficient mice. Although neither Haley et al. (18) nor Ershoff and Steers (12) showed dietary vitamin E to increase survival time in irradiated mice, Sakamoto and Sakka (34) did demonstrate such radioprotection. Other antioxidants were radioprotective in the study by Ershoff and Steers (12).

Radioprotection of the CNS with antioxidants has not been demonstrated previously or in our experiments. Statistically significant differences in levels of vitamin E in the spinal cords of the rats were achieved by varying the amount of vitamin E in their diets over a long interval. Increases in the vitamin E level, however, did not diminish the radiation-induced damage to the CNS in the rats, nor did deficient vitamin E levels result in hypersensitivity to radiation (Fig. 7).

In summary, our data do not support a role for lipid peroxidation in white matter injury after irradiation of the CNS. Although we were interested in whether peroxidation was ongoing during the latent period of CNS radiation injury in general, the spreading low attenuation seen on CT scans after brachytherapy in particular sparked our interest (Fig. 1). Experiments in a dog brain brachytherapy model have shown prolonged focal disruption of the blood-brain barrier in the volume around the implant, combined with progressive leakage of edema fluid into the surrounding interstitial space (14). The dependency on corticosteroids by most patients after brachytherapy (16, 17, 28) substantiates that the appearance of post-brachytherapy CT scans results from spreading edema rather than from spreading peroxidation. We are currently measuring the vascular permeability in the irradiated segment of spinal cord in our rat model, and we are seeking alternatives to corticosteroids for the treatment of radiation-induced edema (15).

REFERENCES


