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RADIATION-INDUCED CHANGES IN THE PROFILE OF SPINAL CORD SEROTONIN, PROSTAGLANDIN SYNTHESIS, AND VASCULAR PERMEABILITY

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Purpose: To investigate the profile of biochemical and physiological changes induced in the rat spinal cord by radiation, over a period of 8 months.

Methods and Materials: The thoraco-lumbar spinal cords of Fisher rats were irradiated to a dose of 15 Gy. The rats were then followed and killed at various times afterward. Serotonin (5-HT) and its major metabolite 5-hydroxyindole-3-acetic acid (5-HIAA) were assayed as well as prostaglandin synthesis. Microvessel permeability was assessed by quantitative evaluation of Evans blue dye extravasation.

Results: None of the rats developed neurologic dysfunction, and histologic examination revealed only occasional gliosis in the ventral white matter at 240 days after irradiation. Serotonin levels were unchanged at 2, 14, and 56 days after radiation but increased at 120 and 240 days in the irradiated cord segments when compared to both the nonirradiated thoracic and cervical segments ($p < 0.01$) and age-matched controls ($p < 0.03$). The calculated utilization ratio of serotonin (5-HIAA/5-HT) remained unchanged. Immediately after radiation (at 3 and 24 h) an abrupt but brief increase in the synthesis of prostaglandin-E_2 (PGE_2), thromboxane (TXB_2), and prostacyclin [6 keto-PGF1α (6KPGF)] was noted, which returned to normal at 3 days. This was followed after 7 and 14 days by a significant fall off in synthesis of all three prostaglandins. Thereafter, at 28, 56, 120, and 240 days, escalated production of thromboxane followed, while prostacyclin synthesis remained markedly reduced (−88% of control level at 240 days). Up to 7 days after radiation the calculated TXB_2/6KPGF ratio remained balanced, regardless of the observed abrupt early fluctuations in their rate of synthesis. Later, between 7 and 240 days after radiation, a significant imbalance was present which became more pronounced over time. In the first 24 h after radiation, a 104% increase in microvessel permeability was observed which returned to normal by 3 days. Normal permeability was maintained at 14 and 28 days, but at 120 and 240 days a persistent and significant increase of 98% and 73% respectively above control level was noted.

Conclusions: Radiation induces severe impairment in microvessel function even in the histologically unaffected spinal cord, and alters the secretory phenotype of various cell systems in the central nervous system.

Prostaglandins, Radiation, Serotonin, Spinal cord, Vascular permeability.

INTRODUCTION

Although the incidence of radiation myelopathy is low (35), the fear of consequent severe neurologic morbidity and a high rate of mortality (38) often leads the radiotherapist to reduce the total radiation doses delivered to tumors located in the vicinity of the spinal cord. It is, therefore, not surprising that radiation injury to the spinal cord elicits a vast clinical and research interest, and the radiation response of the spinal cord has been studied by radiobiologists in almost all available laboratory animal species (35). Considering this level of interest, it is amazing that the interpretation of the pathogenesis of radiation injury to the spinal cord is based entirely on morphological observations and on the analysis of histological specimens using only the most basic staining procedures (i.e., hematoxyline-eosin, myelin, and axonal stains) (35, 51). In experimental models of radiation myelopathy there has been no use of modern immunohistochemical or immunoenzymatic techniques that might convey functionally oriented information in addition to accurate definition of the various cell populations that participate in the evolutions of injury. Recently, immunohistochemical techniques were applied in a mouse model of brain irradiation (3).

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Only two studies have so far been reported to show quantitative nonmorphological evaluations of radiation myelopathy following treatment with large single radiation fractions that ultimately led to paralysis (5, 12). The study that evaluated capillary permeability (5) confirmed the general impression derived from morphological analysis that the blood–spinal cord barrier is disrupted at the late phase after irradiation. The other study evaluated biochemical changes associated with lipid peroxidation (12) and could not confirm the hypothesis that the white matter necrosis seen in radiation injury is related to free radical-induced lipid peroxidation.

The pathologic findings described in radiation myelopathy are almost always confined to the white matter and consist of various combinations of demyelination, gliosis, white matter necrosis, vascular changes, and occasional mononuclear inflammatory responses (5, 35, 37). It is clear that in far advanced injury it is impossible to identify any specific target cells or to recognize the pathophysiological pathways involved in the mechanism of injury. The general view is that oligodendrocytes and endothelial cells are the potential target cells with either independent or overlapping roles in the pathogenesis of radiation damage. It has also been suggested recently that radiation may alter the secretory phenotype of resident astrocytes and microglia, and thereby modify cytokine production (35, 36). Cytokine and other soluble mediators can cause inflammatory response, cellular proliferation, and cellular injury in the central nervous system (CNS), as described in other disease states associated with demyelination (11, 13, 39).

Prostaglandins are a group of biological mediators that have received attention as mediators of radiation injury outside the CNS (2, 34, 50). These compounds have a number of important physiological roles in vasoregulation, smooth muscle regulation, electrolyte balance, and neuroregulation, as well as pathological roles in inflammation and modification of platelet–vessel wall interaction (25, 26, 48). Various components of the CNS may respond to stimuli with increased production of a specific type of prostaglandin: microvessels synthesize prostacycline (PGI_2), and to a lesser extent prostaglandin E_2 (PGE_2), while platelets and astrocytes are the major source of thromboxane (TXB_2) (23). Activated phagocytes or microglia will produce PGE_2, and even biogenic amines (such as neurotransmitters) can stimulate prostaglandin biosynthesis (4, 29).

Various components of the vascular wall have the potential to synthesize prostaglandins. Endothelial cells contain mechanisms for deacylating phospholipids leading to the release of arachidonic acid and formation of prostaglandins (48). Smooth muscle cells possess receptor mechanisms for serotonin type 2 ($5\text{-}HT_2$), that are coupled to arachidonate release in their response to serotonin (4). Mast cells, which are located within the adventitial layer of blood vessels, contain histamine, serotonin, and other biogenic amines that may be released by either chemical, mechanical, or ionizing radiation stimuli and activate receptors that are also coupled to arachidonate release (16, 29). In addition, serotonin is thought to mediate inflammatory reactions leading to thrombosis (54), vasospasm (28), and traumatic extravasation (42). Thus, inflammatory responses and neurotransmitter alterations may be interrelated processes and serotonin may prove to be a vital link in that regard.

In the present study, we evaluated the profile of changes in prostaglandin synthesis, in the levels of serotonin and its major metabolite, and in microvessel permeability in the spinal cord during a follow-up period of 8 months following irradiation. To simulate as closely as possible the clinical use of radiotherapy in humans we chose to give a large dose of ionizing radiation that does not lead to paralysis or to profound tissue damage, but is large enough to cause measurable changes in the vascular permeability and in the presumably allied biochemical parameters.

METHODS AND MATERIALS

Animals and Radiation

Female Fisher rats weighing 150–200 g were anesthetized with pentobarbital (50 mg/kg IP) and positioned back to back lying on the side in a specially designed holding apparatus. The T12–L3 segments were irradiated through an adjustable opening in the lead shield that protected the whole body. Their thoracolumbar spinal cords were irradiated with an orthovoltage machine (175 kv, 0.2 mm Cu, Source-Skin Distance 40 cm) at a dose rate of 1.33 Gy/min. A dose of 15 Gy was administered using a 5×2.5 cm field. Following treatment, the animals and their sham irradiated age-matched controls were weighed weekly and watched closely for any sign of neurological abnormalities.

Measurements of spinal cord serotonin and its major metabolite

The levels of spinal cord serotonin (5-HT) and its major metabolite 5-hydroxyindole-3-acetic acid (5-HIAA) were evaluated in control sham-irradiated animals ($n = 10$) and in irradiated rats ($n = 10$). The irradiated animals and their matched controls were killed at the following time points after irradiation: 2, 14, 56, 120, and 240 days. To eliminate the effect of circadian variation in spinal monoamine content, all sampling procedures were performed at the same time of the day.

Following decapitation under ether anesthesia, the rat spine was immediately cut into four segments: cervical (C1–C7), high thoracic (T1–T6), low thoracic (T7–T12), and thoracolumbar (T13–L3). The spinal cord segments were rapidly removed by laminectomy, frozen on dry ice, and stored at −80°C prior to analysis. After homogenization in 0.1 M perchloric acid, the deproteinized aliquots were assayed for 5-HT and 5-HIAA using a system of high performance liquid chromatography with electrochemical detection.

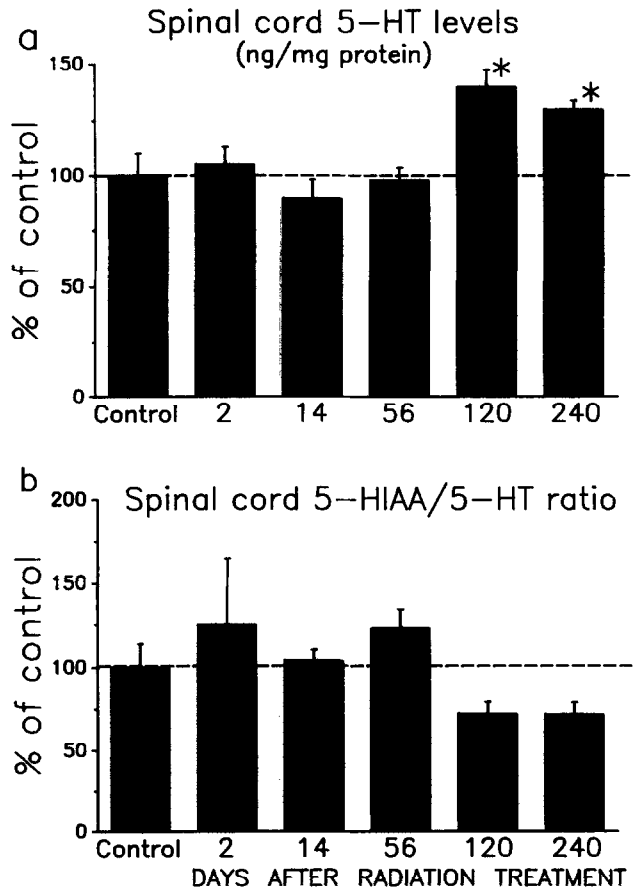

Fig. 1. Levels of serotonin (5-HT) (a) and its utilization ratio calculated as 5-HIAA/5-HT (b) in the thoracolumbar spinal cord of irradiated rats and of age-matched controls. The mean ± SEM levels are presented as percent of their age-matched controls (see text). *$p < 0.03$.

Effect of radiation on spinal cord prostaglandins synthesis

Both irradiated animals and their age-matched controls ($n = 12$ for each group) were killed by decapitation under ether anesthesia at the following time points after irradiation: 3 h, 1, 3, 7, 14, 28, 56, 120, and 240 days. The cervical (C1 to C7) and thoracolumbar cord segments (T13 to L3) were rapidly removed by laminectomy and placed on an ice-cold petri dish. Hand-cut 15 to 20 mg slices were placed in a tube containing 1 mL oxygenated (95% O_2, 5% CO_2) Krebs solution at pH 7.4. The solution was replaced within 1 to 2 min with fresh Krebs sulution and reoxygenated. The tube was tightly closed and placed for 1 h in a shaking water bath at 37°C. At the end of the incubation period, the medium was decanted, stored at −80°C, until assayed for prostaglandins. The slices were homogenized in 1 mL water, and protein content was determined by the Lowry method. The amount of prostaglandins PGE_2, TXB_2 and 6-keto $PGF1\alpha$ (6KPGF) was determined from 0.1 mL of the supernatant that was taken for each radioimmunoassay. For the radioimmunoassay we used ^{3}H-PGs[1] (100–200 Ci/mmol) and specific rat antibodies[2] which had less than 1% crossreactivity with all major prostaglandins. The radioimmunoassay was carried out as previously reported by us (43, 46, 47).

Effect of radiation treatment on spinal cord vascular permeability

Irradiated rats and their age-matched controls ($n = 9$ for each group) were killed at the following time points after irradiation: 1, 3, 14, 56, 120, and 240 days. Twenty four hours before killing, Evans blue (2 mL/kg of 2% dye in 0.9% NaCl) was injected intravenously. Immediately after killing (by an overdose of pentobarbital), the circulation was cleared by transcardiac perfusion of 150 mL 0.9% NaCl at 100 mmHg pressure. The thoracolumbar (T13 to L3) spinal segments were rapidly removed by laminectomy and immediately weighed. A volume of dimethylformamide,[3] two times the spinal cord weight, was added and the tissue homogenized by sonication. The mixture was then incubated in 50°C for 24 h and centrifuged for 20 min at 12000 × g to remove suspended particulate matter. The quantity of extracted Evans blue dye in each supernatant sample was spectrophotometrically analyzed at the absorption maximum for Evans blue in dimethylformamide (635 nm). A standard curve of Evans blue in blank dimethylformamide (extraction of spinal cord tissue without injection of Evans blue) is used to convert absorbance into μg Evans blue/cc dimethylformamide, which is converted into μg Evans blue/g wet tissue weight (30, 56).

Statistical analysis

Differences among groups and locations along the spinal cord are assessed by analysis of variance (ANOVA) followed by the Student–Neuman–Keuls tests. A p-value less than 0.05 is considered statistically significant.

RESULTS

Histopathology

Animals were randomized to their various experimental groups immediately after irradiation. No change in limb function or in tail tonus was observed up to 8 months after irradiation. Histological examination of the spinal cord performed after 120 and 240 days did not reveal any marked abnormalities on hematoxyline-eosin stain, nor white matter changes or blood vessel pathology. Mild gliosis was sometimes noted in the ventral white matter region.

Spinal cord 5-HT and 5-HIAA levels following irradiation (Fig. 1)

Figures 1a and b present the results of 5-HT and 5-HIAA/5-HT ratio (the utilization ratio of serotonin),

[1]Amersham International plc, Buckinghamshire, England.
[2]BioMakor, Rehovot, Israel.
[3](Sigma Chemical Co., St Louis, MO).

measured in the thoracolumbar spinal cord segments of irradiated rats and compare it to the corresponding segments of their age-matched controls. The mean ± standard error of the mean (SEM) values of 5-HT and 5-HIAA/5-HT ratio are presented as percent of control because the normal levels of 5-HT and 5-HIAA are significantly different in older rats when compared to the levels in the younger age group. The normal level of 5-HT in 2- to 3-month-old rats is 6.44 ± 0.63 ng/mg protein (mean ± SEM), while in the 6- to 10-month-old group, it is 4.64 ± 0.54 ($p < 0.03$). The 5-HIAA/5-HT ratio also differs between these two age groups being significantly higher ($p < 0.02$) in normal older rats, indicating that the utilization of 5-HT is either unchanged or enhanced in older rats.

Similar to our previous observations (44, 45), a consistent rostral to caudal gradient for the levels of both 5-HT and 5-HIAA was observed in both normal and irradiated rats, but the calculated ratio of 5-HIAA/5-HT remained constant along the spinal cord of each experimental group, with no significant difference between the four locations (data not presented).

Following irradiation, no significant changes were observed in the levels of 5-HT measured after 2, 14, and 56 days. However, after 120 and 240 days, spinal cord 5-HT levels were significantly elevated ($p < 0.03$) only in the irradiated cord segment as compared to both age-matched unirradiated counterparts (Fig. 1a) and to the nonirradiated thoracic and cervical cord segments ($p < 0.01$). This shift in 5-HT level was not associated with significant change in its utilization (although the calculated ratios are somewhat lower than those of controls (Fig. 1b), signifying that there is an excess of serotonin that is not being used at an accelerated rate.

Effect of radiation on spinal cord prostaglandins synthesis

Figure 2a, b, and c present the temporal changes in the synthesis of PGE_2, thromboxane (measured as TXB_2), and prostacyclin [measured as 6-keto $PGF1\alpha$ (6KPGF)] measured at different time points after irradiation. The results of measurements of all three prostaglandins showed that no significant differences were observed between values obtained at 3 and 24 h after radiation (acute phase), between 7 and 14 days (early postradiation phase), 28 and 56 days (delayed phase), and 120 and 240 days (late-delayed phase). Therefore, the data of the appropriate time points were combined and are presented in Fig. 2 according to these postradiation phases. At each time point, the results are compared to those of unirradiated age-matched controls and are, therefore, presented as the percent change from control values.

It is evident that immediately after radiation (at 3 and 24 h) there is an abrupt increase in the synthesis of all

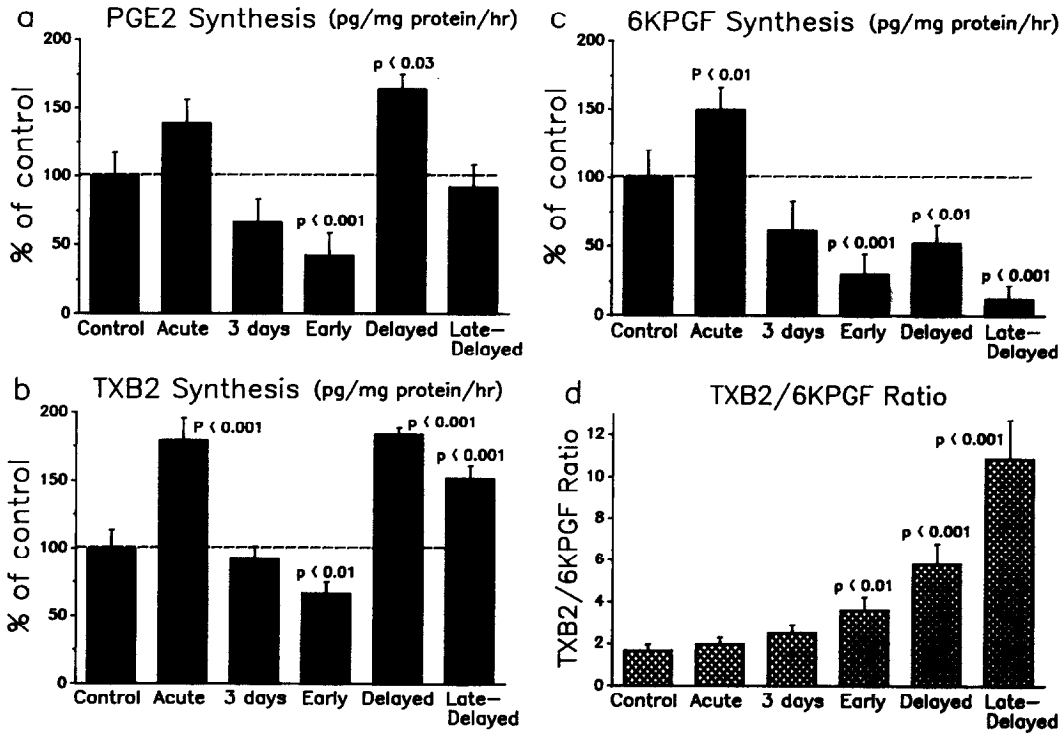

Fig. 2. The profile of synthesis of prostaglandin E_2 (a), thromboxane (measured as TXB_2) (b), and prostacyclin [measured as 6 keto-$PGF1\alpha$ (6KPGF)] (c) in the thoracolumbar spinal cord of irradiated rats and their age-matched controls. Figure 2d present the calculated ratio TXB_2/6KPGF in the spinal cord of irradiated rats and their matched controls. The mean ± SEM levels are presented as percent of matched controls (see text). Results obtained at different time points after radiation are combined as follows: acute phase = 3 and 24 h after radiation, early phase = after 7 and 14 days, delayed phase = after 28 and 56 days, and late-delayed phase = after 120 and 240 days.

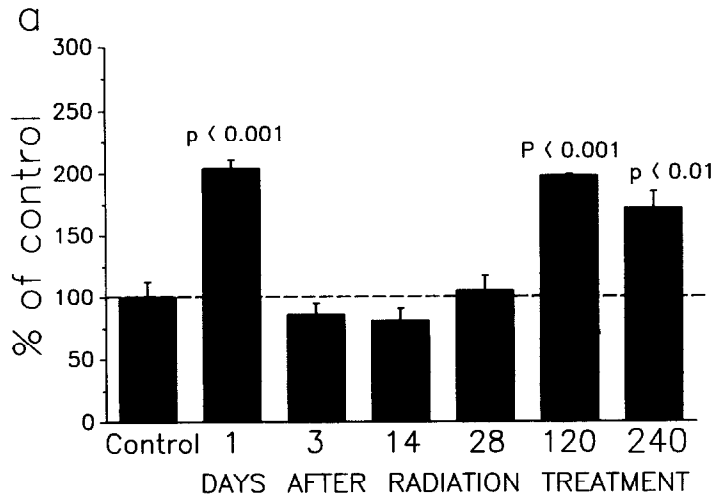

Fig. 3. Spinal cord vascular permeability measured as Evans blue dye extravasation in the thoracolumbar segments of irradiated rats and their age-matched controls. The mean ± SEM levels are presented as percent of matched controls (see text).

three prostaglandins, which is brief and has already normalized at 3 days. Early (7 and 14 days) after radiation, a significant fall off in the synthesis of all three prostaglandins is observed. Later on, the pattern of changes differs for each of the prostaglandins. Escalated production of TXB_2 is noted at the delayed and late-delayed phases after irradiation, while the synthesis of 6KPGF is markedly reduced (being −88% of control level at 240 days).

Figure 2d presents the calculated balance of TXB_2-6KPGF in the spinal cord at each phase after irradiation. It is evident that up to 7 days after radiation exposure, no imbalance in TXB_2-6KPGF could be detected, regardless of the abrupt changes in their levels measured at the acute phase (first 24 h) and 3 days after radiation. However, at 7 and 14 days after radiation, while the rate of synthesis of both prostaglandins was still markedly reduced (Fig. 2b and c), a statistically significant imbalance in TXB_2-6KPGF ratio first appeared ($p < 0.01$). Afterward, as the time after radiation increases, this imbalance became even more pronounced, although it may have been influenced by the exaggerated effect of the small denominator of persistent very low levels of 6KPGF.

Effect of radiation treatment on spinal cord vascular permeability

Figure 3 presents the effect of radiation on dye extravasation in the thoracolumbar spinal segments of treated animals and their age-matched controls. The vascular permeability is approximatly 50% lower in 6- to 10-month-old normal rats ($p < 0.001$) as compared to permeability measured in younger (2- to 3-month-old) animals and, therefore, results are presented as the percent change from age-matched control values.

Twenty four hours after irradiation, a 104% increase in microvessel permeability is observed ($p < 0.001$), but this returns to normal by 3 days. Normal permeability is measured thereafter at 14 and 28 days. At the late-delayed phase after irradiation (120 and 240 days), a persistent and significant shift in permeability is evident with a measured increase in dye extravasation of 98% and 73% above control levels, respectively.

DISCUSSION

Our study clearly indicates that profound early and delayed biochemical and physiological fluctuations arise in a clinically and morphologically intact spinal cord. These changes become extreme as the interval after exposure to ionizing radiation is extended. We demonstrated early and delayed shifts in prostaglandin synthesis and balance, in spinal cord microvessel permeability, and in the concentration of serotonin, but not in its utilization. These measures indicate that a dynamic sequence of events takes place even after exposure to a relatively low dose of radiation that does not lead to clinical or morphological signs of delayed radiation injury to the spinal cord. The parameters we used represent the end products of interaction between multiple cell systems in the CNS and suggest that, as in other tissues (33), a complex interplay of mediators is involved in the pathophysiology of radiation injury.

The role of chemical mediators in altering blood–brain or blood–spinal cord barrier permeability is well established (20, 43, 53). Among these mediators, arachidonic acid and its eicosanoid metabolites have attracted much attention. Under normal conditions, the level of free arachidonic acid in the CNS is negligible (14, 57), but it has been demonstrated that local administration of free arachidonic acid can induce a transient increase in CNS endothelial permeability (27, 52). Arachidonic acid itself (27, 52), as well as its metabolites and their by-products, can disrupt vascular permeability (55). The abrupt but short-lasting increase in prostaglandin synthesis measured in our study at 3 and 24 h after radiation represent the

acute changes that follow irradiation, a profile similar to that which has been described in extra-CNS tissues (6, 10, 16, 32).

Ionizing radiation stimulates the release of free arachidonic acid from membrane phospholipids through activation of phospholipases (16). This causes an abrupt increase in the level of free arachidonic acid in the extracellular space and may, by itself, induce the transient disruption of microvascular permeability measured in the spinal cord of our irradiated rats at the acute phase. At the same time, we also measured a rather nonselective, short-lasting increase in the synthesis of all three prostaglandins assessed by us. According to the method, we used the free arachidonic acid, which is the rate-limiting step in this synthesis, pooled from the irradiated spinal cord. Therefore, the increased production of prostaglandins at that time probably represent the increased availability of free arachidonic acid immediately after irradiation. This newly released free arachidonic acid is used by the specialized enzymes of each cell system and is metabolized into a specific prostaglandin. This may account for the nonselectivity of the increased prostaglandin synthesis and for maintenance of the balance between TXB_2 and 6KPGF in the acute phase in our study. The lack of imbalance between these cyclooxygenase products that have strong antagonistic vasoactive effects, makes it more likely that either the excessive release of free arachidonic acid itself (27, 52) or other by-products (27, 52, 55) of its metabolism account for the transient disruption in micovessel permeability seen in our model. Thus, the abrupt but short lasting increase in prostaglandin synthesis measured in our study at 3 and 24 h after radiation represent the acute changes that follow irradiation, a profile similar to what has been described in extra-CNS tissues (6, 10, 16, 32).

The steep fluctuations observed in the first 24 h after radiation are followed by rapid normalization at 3 days either secondary to exhaustion of the endogenous substrate or due to reduction in the activity of enzymes involved in prostaglandin synthesis. At the early phase after radiation (7 and 14 days), significant reduction in all prostaglandin production is evident in our study. This may be explained either by reduced cell population or by impaired enzymatic activity. It has been demonstrated that following an initial increase, radiation reduces over time the capacity of intact blood vessels and of cultured endothelial cells to produce prostacyclin (1, 10, 32). It was concluded that this is probably caused by damage to the enzymatic mechanism required for prostaglandin synthesis because it could not be corrected by addition of exogenous arachidonic acid or by liberation of arachidonate from membrane phospholipids following stimulation of the membrane phospholipase A_2 (10). Oxygen free radical intermediates, which are formed in our system by ionizing radiation, inactivate the enzymes cyclooxygenase and prostacyclin synthase (9). Recovery from this inactivation is obvious in the spinal cord for PGE_2 and TXB_2 at 28 days after radiation, unlike prostacyclin activity, which is not restored for the entire length of our study up to 8 months after irradiation.

From animal studies it has been postulated that the reciprocal relationship between TXB_2 and 6KPGF is essential for maintenance of cerebrovascular tone and homeostasis (22). Imbalance causes platelets aggregation, microcirculatory stasis and it has also been correlated with the degree of neurological deficit in models of spinal cord trauma (17). A statistically significant TXB_2-6KPGF imbalance is present in our study as early as 7 days after irradiation. This imbalance gradually increases over time, becoming maximal in the late-delayed phase after irradiation. At the early phase, when the enzymatic capacity to produce all three prostaglandins is decreased, the imbalance may result either from the greater sensitivity of prostacyclin synthase to free radical intermediates inhibition (9) or from increased platelet adhesion to endothelial cells with impaired capacity to produce 6KPGF (1, 10). It was demonstrated that irradiation has no effect on platelet thromboxane formation in a dose range of 2 to 20 Gy (1) and, therefore, activated platelets may be the source of excessive thromboxane at this early phase.

The delayed and late-delayed TXB_2-6KPGF imbalance is obviously associated with abnormally enhanced production of thromboxane in face of declining capacity of the irradiated spinal cord tissue to synthesize 6KPGF. In the CNS, platelets and astrocytes are the major source of thromboxane (23). Astrocytes contain receptors for prostanoids and are probably involved in neuro- and immunomodulation through release of eicosanoids and other cytokines (13, 23). The temporary burst of increased synthesis of PGE_2 observed at the delayed phase may suggest that an inflammatory response has been triggered and requires further verification. Nonetheless, in our study, the escalating TXB_2-6KPGF imbalance, which is associated with significant disruption of the blood–spinal cord barrier function, probably suggests a complex pathologic process that involves various components of the vascular wall capable of producing prostaglandins. These components include endothelial cells and astrocytes in capillaries and also smooth muscles in larger vessels. The tissue injury unit includes astrocytes and endothelial cells, and it has been suggested as a sensitive morphological score for moderate radiation injury in the CNS following exposure to single radiation doses in the range of 20 to 25 Gy (31). It is possible that in our model, with the use of even lower doses overt morphological changes are missing, even though functional abnormalities of this unit are clearly present.

The possibility that serotonergic mechanisms are involved in the pathophysiology of CNS radiation injury has never been studied before. We demonstrated a significant delayed increase in the levels of 5-HT in the irradiated segments of the spinal cord. This increase coincides with disruption of microvascular permeability. It is interesting to note that 5-HT is tightly linked to a number of physiological and pathologic processes in the CNS such

as mediation of CNS vascular tone (7, 8), stimulation of release of prostaglandins from vascular and neural tissues (15, 18), and disruption of the blood–brain or blood–spinal cord barrier in some pathological processes (40, 41, 44, 45). We have recently demonstrated that 5-HT mediates its deleterious vascular and clinical effects in a model of neoplastic spinal cord compression through activation of 5-HT type 2 receptors (40, 41). Pharmacological manipulations aimed selectively to block 5-HT_2 receptors significantly improved the function of the disrupted blood–spinal cord barrier in this model, and markedly delayed onset of neurological deterioration. It is suggested that similar pathophysiological mechanisms may operate in this delayed radiation injury model, because there is a temporal association between the abnormal increase in spinal 5-HT levels and the impairment in blood–spinal cord barrier function.

There are several possible sources for the excess level of 5-HT in the irradiated spinal cord: circulating platelets that release their content of stored 5-HT, spinal and perivascular serotonergic nerve terminals, and vascular endothelium or astrocytes that fail to uptake 5-HT by a high affinity mechanism (19, 49). Although the exact source of 5-HT can not be implicated from our study, it seems likely that major contribution may come from circulating platelets. Platelets may interact with the damaged endothelial surface of microvessels that contain cells with impaired ability to synthesize prostacyclin, which is an important component in the vascular defense mechanism. Nevertheless, blockage of 5-HT_2 receptors at both vascular and neuronal sites may be relevant for future neural protection against radiation injury, because these receptors can be located in the CNS vasculature (8, 24), as well as in the intermediolateral and ventral horns of the spinal cord (21), and may be activated by the excessive 5-HT released in their vicinity.

Our study further supports the idea that the asymptomatic interval after radiation is characterized by sequential physiological changes that are imperfectly reflected in routine histological preparations and that even in the histologically unaffected spinal cord, severe impairment in microvessel function is present. This measurable increase in vascular permeability is probably also the end product of a complex pathophysiological process in which many cell systems are involved. Our study also supports the hypothesis that radiation alters the secretory phenotype of various cells in the spinal cord (35), and currently we are evaluating the secretory profile of other mediators that may intervene in radiation-induced CNS injury.

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