



Radiation myelopathy: a radiobiological review

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Summary

Purpose: In radiotherapy the risk of radiation myelopathy is radiobiologically considerable. This review aims to outline future radiobiological research to get closer to the mechanism of radiation myelopathy.

Methods: Different regimes and techniques of irradiation that deliver doses to the spinal cord lead to the development of radiation myelopathy. Up to now, interpretations of injuries have been based entirely on morphological observations and histological analysis.

Results: Using paralysis as the endpoint, large fraction size data were consistent with the linear quadratic model ($\alpha/\beta=2.41$ Gy). Small fraction size data gave a much smaller value of α/β (0.48 Gy).

Conclusion: A possible explanation is the interaction of two different groups of target cells with different fractionation sensitivities and, therefore, different α/β values. This duality suggests that spinal cord vascular endothelial changes, both in the morphology and secretory profile, may convey information in addition to the definition of various cells that participate in the evolution of radiation myelopathy.

Key words: radiobiology, radiation myelopathy, radiosensitivity, immunohistopathology, prostaglandin.

Mielopatia radiacyjna (wywołana promieniowaniem): analiza radiobiologiczna

Streszczenie

Cel: W radioterapii ryzyko wystąpienia mielopatii odgrywa duże znaczenie pod względem radiobiologicznym. Niniejsza analiza ma na celu zarysowanie perspektyw badań radiobiologicznych, w celu lepszego zrozumienia mechanizmu tego rodzaju mielopatii.

Metoda: Różne sposoby działania i techniki napromieniowania rdzenia kręgowego prowadzą do występowania mielopatii. Obecnie interpretacja uszkodzeń oparta jest wyłącznie na obserwacjach morfologicznych i badaniach histologicznych.

Wyniki: Biorąc po uwagę końcowy rezultat, wykazano, że duża część danych jest zgodna z liniowym modelem kwadratowym ($\alpha/\beta = 2.41$ Gy). Na podstawie niewielkiej liczby danych uzyskuje się mniejszą wartość $\alpha/\beta = 0.48$ Gy.

Wnioski: Możliwym wyjaśnieniem jest wzajemne oddziaływanie dwu różnych grup komórek docelowych o różnym stopniu promienioczułości frakcyjnej, a więc o różnych wartościach α/β . Tego rodzaju dwoistość wskazuje, że śródbłonkowe zmiany naczyniowe rdzenia kręgowego, zarówno pod względem morfologicznym jak i ze względu na jego charakter wydzielniczy, mogą dostarczać dodatkowych informacji w zakresie określenia charakteru różnych komórek uczestniczących w ewolucji mielopatii radiacyjnej.

Słowa kluczowe: radiobiologia, mielopatia radiacyjna, promienioczułość, immunohistopatologia, prostaglandyny.

Introduction

Due to the serious risk of morbidity and mortality from radiation myelopathy, the effects of radiation on the spinal cord have become the subject of various studies on in clinical reports.

In spite of a great deal of works, it seems there is a long way to know the basic mechanisms in developing radiation myelopathy and the dose response in the human spinal cord is still poorly defined.

There are many techniques in radiotherapy in which the dose received by the spinal cord is radiobiologically

considerable [1,2,3,64,65,66,67]. The aim of this study is to review previous experiments in order to define what should be done in future radiobiological research to get more familiar with the mechanism of developing radiation myelopathy.

Materials and methods

The majority of experimental studies on spinal cord dose-response have been done on the rat. Different types and methods of irradiation have been used in different animals to study the relative biological effectiveness (RBE) dose response, histopathology, pathogenesis and latency of myelopathy [2,4-8].

Up to now, the interpretation of the pathogenesis of radiation injury to the spinal cord has been based entirely on morphological observations. The use of modern immuno-histochemical or immunoenzymatic techniques that might convey information about the effects of irradiation on the change of the endothelial cell secretory profile in a cultured medium in the central nervous system has become more widespread. *Table 1* shows some of the techniques and methods of assays used by different authors 9-16].

Animals can be irradiated from the lateral aspects with a circular focused beam of 25 mm in diameter applied to the lumbar vertebra (L2-L4). Radiation can also be delivered from an X ray generator with specification of 300 kV at a dose rate 300 rad/minute [17]. For irradiation the animals are fixed to a special jig. Three millimeter thick lead

was used to shield the esophagus, two rats being irradiated at one time placed back to back. An X ray machine operated at HVL = 1.4 mm Cu [18,19].

Boron Neutron Capture Therapy (BNCT) was performed to understand the pathogenesis of late radiation damage to the rat spinal cord [20]. In this study, dosimetric measurements were carried out on dead rats to determine thermal neutron fluence at the skin surface and in the spinal cord.

Due to some specific physical parameters, linacs can be used for irradiation of biological specimens. Irradiation of an umbilical cord cultured medium with dose of 200 cGy was done with clinac 4 MV photons. At this medium was assayed for measuring of prostacyclin (PGI₂) and its metabolite changes with doses comparable to radiotherapy [21-23].

Dosimetry methods

The effects of ionizing radiation in biological systems strongly depend on the dose and related factors such as dose rate, dose distribution, and radiation quality [24]. According to the recommendation of the International Atomic Energy Agency (IAEA 1996) an ionization chamber calibrated for X-rays, based on the Kinetic Energy Released in Medium (KERMA) of the particles produced by radiation, can be used for dose measurements in tissue equivalent phantoms. Using the following equation a dose in water can be obtained

$$D_w = M_{ul} N_k (\mu_{en} / \rho)_{w,a} k_{a,w} ,$$

Table 1. Some methods and materials used by authors.

Assay	Animal	Radiation type	Field size	Dose (Gy)	Authors
Histopathologic	Rat	X-ray & Neutron	2 x 1.5 cm	35	van der Kogel et al., 1974
Histopathologic	Rat spinal cord	X-ray 0.5 mm Cu	4 mm 8 mm 16 mm	18 - 25 20 - 40 25 - 70	Hopewell et al., 1987
Histopathologic	Rat	250 kVp X-ray	Brain	17.5 - 25	Calvo et al., 1988
Histopathologic	Rat	100 kVp X-ray	Cervical spine (2 cm)	24.7 Gy/ /10+top up	Wong et al., 1993
Immunohistochemical	Bovine aorta	230 kVp X-ray	Endothelial cell culture medium	0.05 - 2	Hosoi et al., 1993
Histopathologic	Rat	BNCT	Spinal cord (15 mm)	27 - 35	Moris et al., 1994
Immunohistochemical	Rat	175 kVp X-ray	Lumbar spine (5 x 2.5 mm)	15	Siegal et al., 1995
Histopathologic	Rhesus monkey	-	-	-	Inea et al., 1996
Immunohistochemical	Human umbilical cord	Oxidative stress	Endothelial cell culture medium	No radiation	Estrada-Garcia et al., 2002

where M_w is the dosimeter reading, N_k the air kerma calibration factor ($(\mu_{en}/\rho)_{w,a}$, the ratio of the mean mass energy absorption coefficients of water, and $k_{a,w}$ is a product of the displacement and angular response dependence of the ionization chamber correction factors.

There are other methods of passive dosimetry such as thermoluminescent materials, Fricke dosimeters, photographic films and Diode dosimeters that can be used in various cases and in appropriate conditions.

For the irradiation of small experimental animals it was recommended to use X-rays with a first half value layer, $HVL_1 > 1.0$ mm Cu. However for irradiation of cell cultures on some occasions very low photon energies have been applied with an $HVL_1 < 1.0$ mm Cu [24].

Assay techniques

For histopathological studies routine staining with some differences were used by authors. One of the most standard method is to take 2 mm thick transverse slices from the irradiated spinal cord and stained in Mayer's hematoxylin and eosin or Luxol Fast Blue PAS [18].

Recently, immuno-histochemical techniques were applied in mouse and rat models of the brain and spinal cord irradiation [22, 25].

In a study on radiation-induced changes in the secretory profile of female Fisher rats T12-L3 segments of the spinal cord were irradiated. In this study, samples of spinal cords were analyzed by thin-layer chromatography. Uptake of ^{14}C -labelled arachidonic acid and its conversion to 6-keto-PGF $_{1\alpha}$ (a metabolite of Prostacyclin) were calculated as a percentage of the total arachidonate incorporated [22].

The measurement of prostacyclin (PGI $_2$) production can also be performed by radio-immunoassay (RIA) or enzyme-immunoassay (EIA) of its products such as 6-keto-PGF $_{1\alpha}$ [16].

Immuno-histopathological changes

Theories related to the pathogenesis of radiation-induced CNS lesions/ necrosis fall into two main categories: (a) necrosis due to the death or reproductive sterilization of glial cells, or (b) necrosis caused by vascular damage and the resultant ischaemia. The earliest and most pronounced changes were seen in blood vessels in close association with astrocyte enlargement. This led to the concept of vascular-glial unit of tissue injury (TIU). The incidence and severity of the TIU apparently increased with time after irradiation until the development of necrosis. These dose-related vascular-glial changes were preceded by a reduction in the endothelial cell and vascular density. No early changes were observed in the number of glial cells [20].

The loss of myelin (demyelination) from individual nerve fibers and necrosis (malacia) of groups of nerve fibers are

common expressions of white matter injury, regardless of the etiology. Accordingly, demyelination and malacia are the consistent and dominant morphological features of clinical and experimentally induced radiation myelopathy (Figure 1).



Figure 1. Severe demyelination of the posterior and especially the lateral columns.

Although damage and loss of nerve fibers are inevitable components of lesions in symptomatic cases, the response of the vasculature and glial cells are highly variable and as a result, add controversy to the interpretation of the pathogenesis (1). *Table 2* shows a list of consistent features in human cases and animal models for white matter lesions.

Categorization of radiation myelopathy can be based on the lesions observed in autopsy material [26]. A type I lesion invades only the white matter parenchyma. A type II lesion is predominantly vascular in nature, and type III damage to both of them. The vascular lesions encountered in the irradiated spinal cord are listed in *Table 3*. The vascular changes most often encountered are endothelial alterations, telangiectasia, hyalinosis and fibrinoid necrosis.

Table 2. Vasculopathies of radiation myelopathy.

- | |
|---|
| 1. None |
| 2. Increased vasculopathies |
| 3. Telangiectasis |
| 4. Hyaline degeneration and thickening |
| 5. Oedema and fibrin exudation |
| 6. Perivascular fibrosis and inflammation |
| 7. Vasculitis |
| 8. Thrombosis |
| 9. Fibrinoid necrosis |
| 10. Haemorrhage |

Although haemorrhagic necrosis is listed as a white matter lesion, thrombosis and vascular necrosis are essential parts of the process (Figure 2).



Figure 2. Demyelination and axon loss in motor and sensory columns especially in the lateral corticospinal tracts. Injured regions contain a prominent concentration of small blood vessels.

From the pathological view, findings 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 inflammatory responses. It is clear that in advanced injury it is impossible to identify any specific target cells or to recognize the pathophysiological pathways involved in the mechanisms 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 structures which mimics other diseases that are the pathological evidence of radiation myelopathy [1, 27, 28].

Study of the dose- effect relationship showed that the largest dose at which no symptoms of myelopathy were obser-

ved within one year was about 19 Gy for X-rays. Histological observations of this study showed that irradiation with doses that are a few hundred cGy's or more in excess of the tolerance level induces a uniform histological picture. Three months after irradiation a slight degeneration of the nerve roots was seen. Myelin sheaths became smaller in diameter after 5-6 months. The spinal roots were partly or totally necrotic. There were no changes in the white matter and in the grey matter. Vascular damage was scant and consisted of hyalinisation and fibrinoid thickening of the vessel walls with constriction of the lumen [17].

Recent immuno-histochemical techniques in rat models to evaluate the pathogenesis of radiation myelopathy reveal changes in the secretory profile of the spinal cord. However, no significant changes were seen in the levels of 5-HT (serotonin) after 2, 14 and 56 days. But, after 120 and 240 days, the spinal cord 5-HT levels were significantly elevated. It was also evident that immediately after irradiation (at 3 and 24 hours) there was an abrupt increase in the synthesis of all three prostaglandins (PGE₂, thromboxanes A₂ and prostacyclin). Early (7 and 14 days) after radiation, a significant fall - off in the synthesis of all three prostaglandins was observed. Later on, the pattern of changes differed for each of the prostaglandins [22].

The study of the effects of radiation on biological functions the effect of radiation on suppression of PGI₂ production showed that irradiation with 25 cGy had a tendency to decrease PGI₂ production. The degree of suppression was almost constant irrespective of the concentration of arachidonic acid, and it was 39% of control at 100 ng/ml of arachidonic acid. The PGI₂ production decreased with a maximal decrease at 100 cGy and without further decrease at 200 cGy. PGI₂ productions at 24 and 48 h after irradiation did not differ from central levels. Therefore, the suppressive effect seemed to last only a short period of time after irradiation [9, 10].

Prostaglandins are a group of biological mediators from unsaturated fatty acids that have recently been shown as mediators of radiation injury outside the CNS. Prostaglandins' synthesis involves the cyclooxygenase pathway as shown in Figure 3.

These compounds have a number of important physiological roles in vasoregulation, smooth muscle regulation, electrolyte balance and neuro-regulation as well as in the

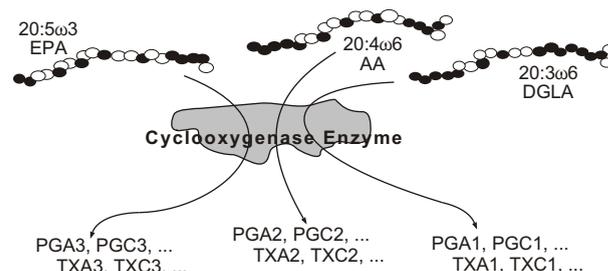


Figure 3. Fatty acid relationships to eicosanoid formation.

Table 3. White matter lesions of radiation myelopathy.

1. Demyelination of isolated nerve fibers
2. Demyelination of groups of nerve fibers (Spongiosis)
3. "Inactive" malacia
a) Spongiosis, spheroids
b) Scar
4. "Active" malacia
a) Coagulative malacia
b) Liquifactive malacia
I) Amorphous
II) Foam cell fields
III) Cystic
5. Haemorrhagic malacia

pathological role in inflammation and modification of platelet-vessel wall interaction. Thus inflammatory responses may be related to the changes in the levels of prostaglandin production. Various components of the CNS may respond to stimuli with increased production of a specific type of prostaglandin [22, 29, 30].

Prostaglandin I₂ as called **Prostacyclin**, is produced primarily in the endothelial linings of blood vessels and has been found to be lower in conditions associated with vascular insufficiency and microangiopathy. Allen et al 1982 demonstrated that after a radiation dose of 200 cGy in vitro, endogenous bioassayable PGI₂ activity was very much decreased or absent. Vascular cyclo-oxygenase and prostacyclin synthase are sensitive to inhibition by lipid peroxides. It is possible therefore that lipid peroxides may be formed during vascular irradiation and may cause inhibition of PGI₂ production. Since PGI₂ is a potent vasodilator and anti-thrombotic agent, these pathological vascular changes may be explained by the inhibition of PGI₂ production [23, 29, 30, 31, 32, 33, 60].

Radiobiological stand points

Because of the morbidity of radiation myelopathy, spinal cord doses should always be minimized. However, to the authors knowledge no myelopathies have been reported using 1.2 Gy fractions given twice daily to a total dose of approximately 45 Gy and with the interval no shorter than 6 hours [2, 17].

Each radiobiological study needs a strict end effect caused by irradiation. End points of spinal cord irradiation were characterized as paralysis of both fore- and hind limb according to the size and dose of radiation.

The mean latency for developing radiation damage to the spinal cord clearly declined with increasing doses, reaching a value of < 30 weeks for doses of ≥ 23 Gy, 40 Gy and ≥ 54.5 Gy for fields 16, 8 and 4 mm in length respectively. A mean latency of 60 weeks was associated with doses of ~20.5 Gy, ~24 Gy and ~40 Gy for 16.8, and 4 mm fields, respectively.

When animals survived for > 30 weeks there was a considerable variation in the latency. However, the mean latency appeared to be linearly related to the dose for the three different lengths of the spinal cord irradiated.

The dose required to produce paralysis in 50% of rats (ED₅₀) increased from 21.5±0.3 Gy for a 16 mm field to 50.98±2.28 Gy for a 4 mm field. There is no difference in ED₅₀ between two types of histological changes when the field size is 16 mm even under and / or above the 30 weeks after irradiation.

Animals that developed paralysis within 30 weeks had white matter necrosis without damage to the grey matter or nerve roots. Those developing paralysis from between 37 weeks and 99 weeks after irradiation showed a varying severity of telangiectasia in grey and white matter. Demyeli-

nation and nerve root necrosis were also seen with doses ≥ 24 Gy and ≥ 30 Gy the spinal cord of 8 mm and 4 mm in length respectively.

In animals that developed paralysis with a latent period of < 30 weeks very extensive white matter necrosis was seen in dorsal, lateral and ventral columns. The severity was apparently greater than that usually associated with doses of 21-24 Gy to a 16 mm long spinal cord [18, 34].

Modeling

For fitting the obtained data to the linear quadratic (LQ) model, the α/β values at moderate to high doses were significantly greater than the corresponding values at low doses. It was reported that biologically, substructure radio-sensitivity variations can be responsible for under-estimation of the low dose effect from high doses. At even lower doses, i.e., under 1.0 Gy, there is evidence of a different substructure with a paradoxical increase in in-vitro radio-sensitivity. Using paralysis as the endpoint, results from large fraction sizes, i.e. 19 Gy down to 2 Gy / fraction, were consistent with the linear quadratic (LQ) model and α/β values of 2.41 Gy. Data from experiments with small fraction sizes, i.e. 2 Gy down to 1 Gy/ fraction, however, gave a much smaller value of 0.48 Gy for α/β . It is may be concluded that the LQ model failed to provide a satisfactory description of the dose - fractionation response relationship in rats spinal cord [35,36]. This problem is of considerable significance during radiotherapy in clinical situations, since spinal cord shielding is usually introduced toward the end of the course of treatments after the cord has received a near tolerance dose, where only low doses exist. Some of the α/β ratios from different experimental studies in various regimes based on histo-pathological findings are shown in *Table 4*. This duality was explained by the summation of the effect on two target populations in the CNS. A two - cell population LQ model

$$E = -\ln \{ \rho \exp(-\alpha_1 nd - \beta_1 nd^2) + (1 - \rho) \cdot \exp(-\alpha_2 nd - \beta_2 nd^2) \}$$

was fitted to the combined data using the direct method. In this model, ρ and $1 - \rho$ are the respective fractions of the overall effect contributed by the damage to the two target cells [35, 68-71].

Other factors affecting responses

Different reports have shown that the severity and development of radiation myelopathy depend on the type and dosage of the chemical agent administered to the patients, and even more so when it is infused intrathechally.

Because intrathecal chemotherapy alone is quite capable of producing myelopathy, the combination of radiation and intrathecal chemotherapy must be considered to be extremely hazardous [37-44].

Table 4. α/β values of spinal cord from different experimental studies for early- and late-respond.

Spinal region studied	α/β ratio (Gy)	Authors
Cervical spinal cord	1.6 - 1.9	White and Hornesy, 1978
Lumbar spinal cord	4.1 - 4.9	White and Hornesy, 1978
Lumbar	3.7 - 4.5	Van der Kogel, 1979
Cervical	1.8 - 2.7	Van der Kogel, 1979
Spinal cord myelopathy	> 3.5	Dische et al., 1981
Lumbar	3.8 - 4.1	Leith et al., 1981
Lumbar	2.3 - 2.9	Amols and Yuhas (quoted by leith et al., 1981)
Cervical	1.5 - 2.0	Ang et al., 1983
Cervical	2.2 - 3.0	Thames et al., 1988
Cervical;		Ang et al., 1992
mono-exponential repair kinetic model	2.2	
bi-exponential repair kinetic model	2.0	
Spinal cord;		Guttenberger et al., 1992
Overnight-interaction model	2.0	
Cervical;		Wong et al., 1993
Initial top-up dose	0.97	
Final top-up dose	1.23	
Cervical;		Wong et al., 1997
Very small doses per fraction	1.2	
Cervical;		Wong et al., 1997
Retreatment schedule	3.0	
Lumbar	1.46 - 1.12	Pop et al., 1997
Cervical	2.0	Landuyt et al., 1997

Treatment in hyperbaric oxygen (HBO) may reduce the tolerance of the spinal cord to radiation. The clinical data related to blood pressure are somewhat ambiguous and the experiments are very few. Certainly, many reports contain cases in which hypertensive patients have developed radiation myelopathy [45,46,66].

Conclusions

It was revealed that, the LQ model does not provide an adequate description of the dose - fractionation response relationship below 2Gy per fraction of the rat spinal cord. The α/β value decreases with decreasing doses per fraction, and extrapolating the LQ formulation from large fraction size data underestimates the sparing effect of small doses per fraction when a 24 h interval is allowed between fractions for repair of sublethal damage.

Early injury may appear two weeks after completion of irradiation. More often, the evidence of injury is recognized only several months later. Four to five months after irradiation, small vessels show wall thickening with fibrin lesions of the vessel. Endothelial cell proliferation, fibrin thrombi, and perivascular edema with fibrin exudates are

present, and telangiectasias are developing. Veins as well as arteries are damaged; indeed veins may be more severely injured.

Delayed spinal cord injury may be found from 6 months to many years after irradiation. White matter tumour-like masses of cord necrosis are present and spongy-form degeneration and vascular lesions develop [47].

Pathogenesis of the damage to the spinal cord is multifactorial. Direct parenchymal cell radiation injury, with loss or reduction of the function of oligodendroglia, is also involved in the demyelination process. Damage to the astrocytes, and disruption of their anatomic and functional relationship to blood vessels and capillary permeability, leads to edema and fibrin exudation and is important in the formation of coagulative, fibrin-rich necroses. Microvascular damage that reduces blood flow is important. Arteriolar, small arterial, venule, and small vein injuries further compromise blood flow.

Recent immuno-histochemical techniques revealed a possible role of shift in chemical mediators secreted by the spinal cord and supportive structures post-irradiation. Ionising radiation stimulates the release of free arachidonic acid from membrane phospholipids, and its eicosa-

noid metabolites which are of major concern. Arachidonic acid itself, as well as its metabolites and their by-products, can disrupt vascular permeability [22, 48-50].

At an early phase after irradiation (7 and 14 days), significant reduction in all prostaglandin production was evident. This might be explained either by reduced cell population or by impaired enzymatic activity. It was demonstrated that following an initial increase, radiation reduces over time the capacity of intact blood vessels and of cultured endothelial cells to produce prostacyclin. It may be concluded that this might be due to the damage to the mechanism of prostaglandin production [12, 13, 23, 51-59, 72].

The role of prostacyclin level changes in the development of different pathological conditions will undoubtedly become elucidated in the future. Inactivation of the prostacyclin receptor gene results in an increased susceptibility to thrombosis in vivo [61-64].

Blood vessels and the endothelial cells in particular, are characterized as critical targets for irradiation. The duplication of pathology seen after uniform irradiation using conventional treatment modalities, such as X rays, suggests that vascular endothelial cells may be the primary target cell irrespective of irradiation modality.

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