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Time Dependent Repair of Radiation Damage in the Rat Spinal Cord After X-Rays and Neutrons

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Abstract—*The increases in total dose required to cause paralysis after irradiation of the cervical cord or lumbar cord by two equal doses of X-rays or neutrons separated by times from* 1 *to* 120 days *have been measured. No increase was needed until the doses were separated by times in excess of* 15 days. *The timing was the same for irradiation of the lumbar and cervical cord in the same rat strain and was different from that observed in a different rat strain. The time course of repair was the same after X rays or neutrons. The data show that for irradiation induced myelopathy the exponent of T in the Ellis formula is zero until* 15 days *and is then, between* 15 *and* 120 days, 0.08 *for both X-rays and neutrons. Comparison with results of Van der Kogel indicate that the time for which the exponent of T is zero varies with rat strain. It is suggested that the time dependent repair in the spinal cord, which is the same after X-rays or neutrons, is due to repopulation and not slow repair. The recovery from sublethal damage after neutrons is about half that observed after X-rays.*

INTRODUCTION

The inclusion of spinal cord in a radiation field severely limits the treatment dose in radiotherapy. Irradiation of short lengths of the spinal cord of rats with single doses of more than 2000 rad of X-rays results in necrosis of white matter in the cervical region or of the spinal roots in the lumbar region in the year following treatment [1, 2]. After lower doses, damage to blood vessels leads to degeneration between 1 and 2 yr post-irradiation [3].

Most tissues show at least two repair processes. One, usually associated with Elkind repair of sublethal damage, takes a few hours. This may be followed by further repair, attributed to repopulation or "slow repair". Both the lumbar region and the cervical region of the spinal cord possess a large capacity for the repair of sublethal irradiation damage. Experiments in which the cord was exposed to two equal doses of X-rays separated by different periods of time have shown that considerable repair takes place during the first 24 hr after irradiation, values of $D_2 - D_1$ being 6 Gy for the cervical cord [4] and 9 Gy for the lumbar cord [2]. This large capacity to repair sublethal damage is reflected in the large exponent of N in the Ellis formula [5] which relates total dose to the number of fractions and the overall treatment time. Making no adjustment for differences in overall treatment time (T), Van der Kogel [6, 7] found that the slope of the isoeffect curve from 1–20 fractions to the cervical cord was 0.44 and for the lumbar cord was 0.42 and we found a slope of 0.40 for 1–30 fractions to the lumbar cord [2]. The exponent of N derived from these values is high, that measured in the latter study for fractionated doses given in the same overall treatment time of 6 weeks was 0.35 and for 4–60 fractions in 6 weeks was 0.33 [8]. The early recovery from sublethal damage is followed by a period during which no further recovery takes place. In the lumbar cord this period lasts at least 15 days but by 32 days after irradiation, a second but much slower phase of recovery is in progress [2]. Similar experiments on the cervical cord [4] revealed that this latter wave of recovery could not be demonstrated until at least 16 weeks [4].

It might not be surprising for lumbar and cervical cord to show different patterns of recovery from irradiation damage since different populations of cells appear to be involved in the expression of radiation damage in the two regions. In the lumbar region myelin is lost from the axons in the nerve roots presumably due to damage to the Schwann cells whereas in the cervical regions, necrosis of the white matter of the cord itself occurs, the oligodendroglia and vascular endothelium having been suggested as the most vulnerable cell types [6, 7].

In this paper we report an extension of our study on the effects of overall treatment time on the sensitivity of the spinal cord to X-irradiation, this time on the cervical cord for treatment times up to 120 days. The aim of this experiment was to investigate whether the patterns of repair and recovery are similar to those which we have seen in this strain of rats in the lumbar cord or whether they follow the rather different timing found by Van der Kogel [4] in the cervical cord of a different strain of rats. The pattern of recovery in the lumbar cord after irradiation with neutrons is also reported and compared with that after X-irradiation of the lumbar and cervical cord. The implications of this comparison between the effects of neutrons and X-rays on the type of time dependent repair occurring in the spinal cord are discussed.

MATERIALS AND METHODS

The X-irradiation procedures for both the lumbar and cervical cord have already been described in detail [2]. Briefly, the cords of male CFHB rats (aged 3 months and weighing 350–400g) were irradiated using 250 kV X-rays with HVL 1.3 mm Cu. The dose-rate was 2 Gy/min. A lateral beam was used and the length of the cervical cord irradiated was 1.5 cm (C2–C4). In previous experiments the last 2.5 cm of cord (L4–L6) were irradiated. Rats received either a single dose or two equal doses separated by 1, 15, 60 or 120 days. The rats were lightly anaesthetised during irradiation with sodium pentobarbitone (30 mg/kg i.p.). The endpoint chosen for these experiments was ataxia or weakness or loss of use of one or both front legs and this is referred to subsequently, for brevity, as paralysis.

Neutrons were obtained from the MRC Cyclotron at Hammersmith. The lumbar cord was irradiated. The last 2.5 cm of cord were irradiated (vertebrae L4–L6). The endpoint used was the loss of the extension reflex in the hind limbs and ataxia leading to loss of use [2] and again subsequently called paralysis. The neutrons used were produced by bombarding a beryllium target with 16 MeV deutrons producing a horizontal beam of neutrons with a mean energy of about 7 MeV. The neutron beam was collimated by means of paraffin wax and finally by brass tubes lined with steel to give a field size of the same diameter as that used with X-rays. No build up material was used. The dose-rate was about 0.7 Gy/min. The neutron dosimetry has been described elsewhere [9, 10]. The doses quoted are neutron dose only. There is about 3% γ-ray contamination in the beam also. The doses were given as single doses or two equal doses separated by 1, 16, 32 or 73 days.

After irradiation with X-rays or neutrons of either the cervical or lumbar cord, paralysis developed between 4 and 8 months. The endpoint was taken at 1 yr after treatment. For the longer times between fractions of 15–120 days, the mid-point of the overall treatment time was taken to be the first day after irradiation.

RESULTS

Dose–effect curves for X-irradiation of the cervical cord are shown in Fig. 1 and for neutron irradiation of the lumbar cord in Fig. 2. The curves were fitted by eye; the ED_{50} values with the variance were determined graphically as previously described [2]. The doses required to cause paralysis of the front or hind legs in 50% of rats (ED_{50}) at 1 year after irradiation and the variance are shown in Table 1. The statistical procedures have been described previously [2]. Also shown in Table 1 are the ED_{50} values after X-irradiation for the lumbar cord obtained previously. These latter observations unfortunately were terminated at 6 months because of lung infections.

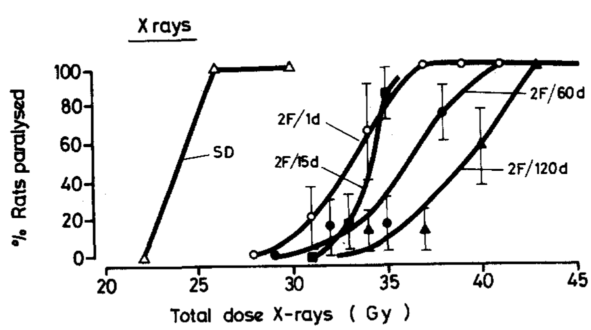

Fig. 1. The percentage of rats showing paralysis of the fore limbs 1 yr *after X-irradiation of the cervical spine.* (△) *Single doses.* (○) *two equal doses separated by* 1 day, (■) *two equal doses separated by* 15 days, (●) *two equal doses separated by* 60 days, (▲) *two equal doses separated by* 120 days.

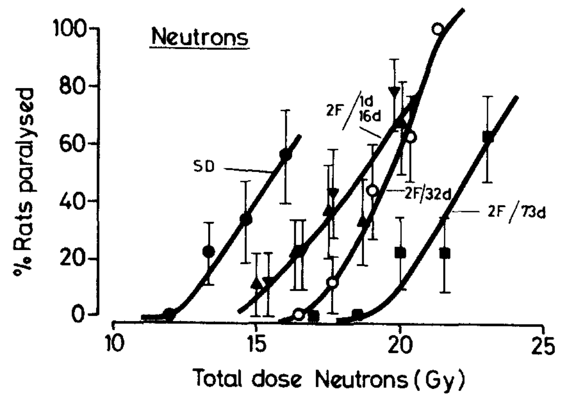

Fig. 2. The percentage of rats showing paralysis of the hind limbs 1 yr *after neutron irradiation of the lumbar spine.* (●) *Single doses,* (▲) *two equal doses separated by* 1 day, (▼) *two equal doses separated by* 16 days, (○) *two equal doses separated by* 32 days, (■) *two equal doses separated by* 73 days.

but the second wave of repair occurs later in Van der Kogel's animals. In our rats this second slower wave of repair starts between 16 and 32 days for the lumbar cord and between 15 and 60 days for the cervical cord whilst the repair started between 8 weeks (56 days) and 16 weeks (132 days) in the cervical cord of Van der Kogel's rats. It can be seen in Fig. 4, where the data on the cervical cord reported in this paper and that for the lumbar cord in the same rat strain previously reported [2] are plotted together, that the timing of this second phase of recovery is similar in both regions of the spinal cord. This suggests that the difference in timing reported by Van der Kogel from that reported here is due to strain differences rather than differences between lumbar and cervical cord.

Table 1.

Site	Radiation	Fractional schedule	ED_{50}(Gy) (±2 × variance) (6 months)	D_T/D_1	D_T/D_2	ED_{50}(Gy) (±2 × variance) (1 year)	D_T/D_1	D_T/D_2
Cervical	X-Rays	Single dose	24.0 ± 2.0	1.00		24.0 ± 2.0	1.00	
		2F/1d	35.5 ± 1.2	1.48	1.00	34.2 ± 0.5	1.43	1.00
		2F/15d	35.5 ± 1.2	1.48	1.00	33.2 ± 1.3	1.38	0.97
		2F/60d	38.0 ± 1.4	1.58	1.07	36.4 ± 1.5	1.52	1.06
		2F/120d	39.8 ± 1.4	1.66	1.12	39.3 ± 1.8	1.64	1.15
Lumbar	X-Rays	Single dose	24.0 ± 0.8	1.00				
		2F/1d 1st expt	33.5 ± 1.0	1.40	1.00			
		2nd expt	33.0 ± 1.0	1.38	0.99			
		2F/4d	32.0 ± 2.4	1.33	0.96			
		2F/8d	32.5 ± 2.0	1.35	0.97			
		2F/16d	32.5 ± 2.0	1.35	0.97			
		2F/32d	36.5 ± 2.0	1.52	1.09			
Lumbar	Neutrons	Single dose				15.5 ± 0.9	1.00	
		2F/1d				18.8 ± 1.5	1.21	1.00
		2F/16d				18.8 ± 1.5	1.21	1.00
		2F/32d				19.5 ± 1.0	1.26	1.04
		2F/73d				22.3 ± 1.0	1.44	1.19

D_1, Total radiation dose delivered in a single fraction.
D_2, Total radiation dose delivered in two equal fractions separated by 24 hr.
D_T, Total radiation dose delivered in two equal fractions separated by various time intervals.

DISCUSSION

Recovery from X-irradiation damage in both cervical and lumbar cord falls into two distinct phases—a rapid phase during the first 24-hr attributable to Elkind repair of sublethal damage and a slower phase starting a few weeks later. Our results and those of Van der Kogel [4] both on the cervical cord, are compared in Fig. 3. Elkind repair has the same time course for both groups of animals

In some tissues, for example the lung, a "slow repair" has been observed immediately following Elkind repair [11]. About half the "slow repair" in the lung is completed within 10 days of X-irradiation. "Slow repair" was absent after neutrons [12]. It is clear from the results reported here that "slow repair" does not occur in the spinal cord. The pattern of the second phase of repair in the spinal cord suggests it may be due to cell regeneration following injury. Injury usually triggers the

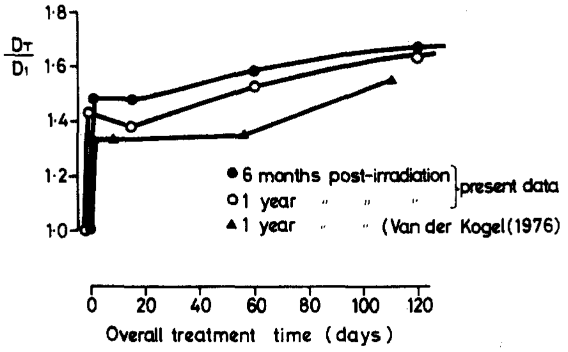

Fig. 3. The ratio of ED_{50} *values for irradiation to the cervical cord given in two doses* (D_T) *and that given in a single dose* (D_1)*, shown in relation to the time between doses (overall treatment time) for observation at* 6 months *and* 1 yr *obtained from the present data and at* 1 yr *from that of Van der Kogel* [6] *on a different strain of rats.*

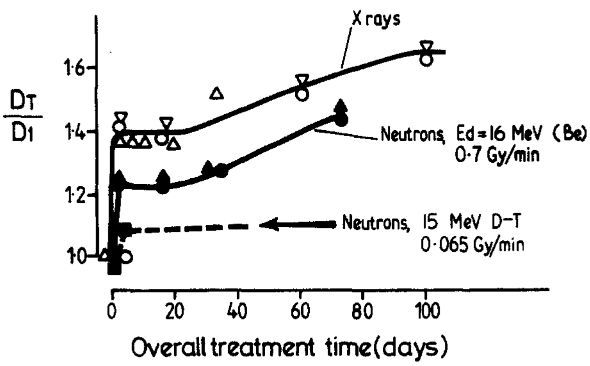

Fig. 4. The ratio of dose given in two fractions (D_T) *to that given in a single dose* (D_1) *vs overall treatment time for rats irradiated with* 250 kVp-rays *or* Ed = 16 MeV(Be) *neutrons to the lumbar or cervical cord reported in this paper and previously* [2] *and the value estimated from Van der Kogel's data* [1, 6] *for* MeV *D-T neutrons.*

homeostatic stimulus to division in cell populations which may normally have low turnover rates. Although damage to the cord is not expressed in terms of ataxia or paresis until at least 100 days after irradiation, Mastaglia *et al.* [13] have shown that Wallerian-type degeneration of axons in the cervical cord occurs as early as two weeks after doses of X-rays as low as 5 Gy. This may stimulate division of oligodendroglia or Schwann cells. Experiments designed to investigate this possibility are now in progress.

In Fig. 4 we have compared the pattern of recovery in the CFHB rats after neutron irradiations with that observed after X-irradiation. The Elkind recovery observed in the first 24 hr is less after neutron than after X-irradiation, but the timing and magnitude of the second phase of recovery are similar after both X-rays and neutrons. The similarity of this second phase of recovery after both X-rays and neutrons gives added support to the suggestion that it is due to repopulation following a homeostatic stimulus to regeneration.

The D_2-D_1 value of 10 Gy observed for X-irradiation of the cervical cord is similar to the D_2-D_1 value of 9 Gy observed for the lumbar cord [2] in this strain of rats, and is consistent with the very large capacity to repair sublethal damage in the spinal cord. These large values of D_2-D_1 give a value of 0.42 for the proportion of dose recovered per fraction $[(D_2-D_1)/D_1]$ for X-irradiated spinal cords. The D_2-D_1 value of 3.2 Gy for neutron irradiation indicates a moderate ability to repair sublethal damage from cyclotron neutrons and gives a value of 0.21 for the proportion of dose recovered per fraction. It can be seen in Fig. 4 that about half as much recovery from sublethal damage occurs after cyclotron neutrons as after X-rays. This is similar to observations on other tissues [14]. This observation was surprising in view of Van der Kogel's results with 15 MeV D–T neutrons [1, 6]. He found a D_5-D_1 value of under 2 Gy. If all this recovery occurred between the first two fractions it would correspond to about 28% of the recovery seen in his animals with X-rays compared with about 50% seen for cyclotron neutrons in this study. The recovery after D−T neutrons is compared with that after cyclotron neutrons in Fig. 4. In other tissues more sublethal damage is seen with 15 MeV D−T neutrons than with ED = 16 MeV(Be) neutrons [15, 16]. It is probable that the reduced repair observed by Van der Kogel is due to the low dose-rate of neutrons used. He used 0.065 Gy/min compared with 0.7 Gy/min used in the present experiments.

In Fig. 5 is shown the isoeffect curve for cord damage leading to ataxia, loss of reflex and paralysis related to overall treatment time for both X-rays and neutrons obtained from data presented in this paper on the cervical and lumbar spine and from that presented previously on the lumbar spine [2] all in the same rat strain. The ratio of the ED_{50} dose given in a number of days (D_T) to that given in 1 day (D_2) is plotted on a logarithmic scale as a function of the interval between fractions also plotted on a logarithmic scale. There is no increase in total dose required as the time is increased until at least 15 days after treatment and it then rises with a slope between 15 and 120 days of 0.08. This final slope is similar to that observed for radiation pneumonitis of 0.07 [11]. However, in lung the recovery process which results in the decrease

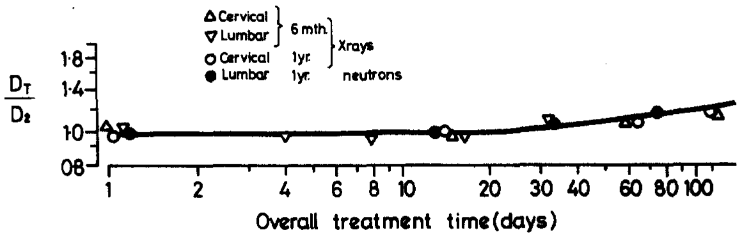

Fig. 5. The isoeffect curve for radiation induced myelopathy after X-ray or neutron irradiation of the cervical or lumbar cord in a single strain of rats shown as a function of the overall treatment time.

of damage by increasing the overall treatment time begins within the first few days following the start of treatment with X-rays, and does not occur after neutrons. The slope of the time-dependent isoeffect curve gives the exponent of T in the Ellis formula. For radiation myelopathy the exponent of T is zero for a period which may depend on animal strain. This means that no accurate exponent for T for radiation myelopathy can be given for use in radiotherapy for it may vary from individual patient to patient. Comparing the increase in total dose needed when given in an overall treatment time of 8 weeks only, obtained here and by Van der Kogel it seems likely that for treatments given in 6–8 weeks the exponent for T will be between 0.00 and 0.03.

If the above observations are applicable to man they indicate that over a normal fractionation schedule used in radiotherapy of about 4–8 weeks there would be almost no sparing of damage attributable to time dependent repair for neutron or X-irradiations. There would, however, be considerable sparing from the fractionation of X-ray dose, the exponent of N in the Ellis formula being about 0.4 [2, 4]. To obtain any additional sparing by extending the overall treatment time, treatment would need to be very protracted or given, perhaps, in a split course extended some months for both X-rays or neutrons.

CONCLUSIONS

(1) Time dependent repair in the spinal cord is probably due to repopulation and not to "slow repair".
(2) The time course of the repair is similar for lumbar and cervical cord but may be rat strain dependent.
(3) Recovery from sublethal damage after neutrons is about half that after X-rays.
(4) The time course of the second phase of repair after neutrons is the same as that after X-rays.

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